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## ERRATA

Vol. XXVI, No. 12, page A228

The first sentence in abstract 623 should read: "This investigation deals with the vaccinator, a vaccine pre-treatment factor in the butter making, developed in New Zealand."

Vol. XXVII, No. 3, page A35

Line 11 in abstract 70 should read: "The phage infection usually occurs from the 'whey fog' from a whey separator. . . ."

Vol. XXVII, No. 4, page A79, line 15

Conclusion 6 should read: "It cannot be safely assumed: . . ."

Vol. XXVII, No. 5, page 516

The last line in the first paragraph should read: ". . . 34.0-49.0 mg protein and 0.57-0.86 mg lipide phosphorus were adsorbed per . . ."

Vol. XXVII, No. 12, pages 851, 853 and 855

Running headlines should read: "VITAMINS IN SUMMER MILK."

Vol. XXVII, No. 10, pages 850 and 851

Running headlines should read: "ARTHUR D. THOMAS ET AL."

Vol. XXVII, No. 10, page 812

Table 1, column 3: 0.151 should read 0.0151.

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# JOURNAL OF DAIRY SCIENCE

VOLUME XXVII

JANUARY, 1944

NUMBER 1

## THE RÔLE OF SURFACE-ACTIVE CONSTITUENTS INVOLVED IN THE FOAMING OF MILK AND CERTAIN MILK PRODUCTS. I. MILK PROTEINS

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### INTRODUCTION

The study of the foaming problem is of considerable interest in the dairy industry as well as several other industries. Some practical problems such as churning of cream, shrinkage of ice cream, whipping of cream or condensed milk, prevention of milk solids losses in manufacturing processes, foaming of casein in paper-coating, and the efficiency of pasteurization are directly related to the foam problem.

The surface-active constituents of milk contributing to its foaming properties, in all probability, are involved also in other physico-chemical problems such as creaming of milk, rebodding of cream, insolubility of milk powders and in the various activities at the fat globule interface.

A survey of the literature indicated the desirability of undertaking a systematic study of the fundamental foam problem in milk products. In the present paper, the apparatus used in these studies and the units of foam measurements are described, and the foaming properties of the major milk proteins are reported.

### REVIEW OF LITERATURE

At the beginning of the present century, Siedel and Hesse (40) presented quantitative proof of a protein accumulation in the milk foam. This work, confirmed later by others (20, 38) marked the start of the present state of confusion as to what protein is responsible for the foaming of milk. Siedel (39) introduced the notion that the foaming substance in milk is other than its two major proteins—casein and albumin. Subsequent efforts were directed towards the finding of the foaming substance in the minor fractions of milk proteins.

Rahn (31) claimed that milk contains a special foam compound, which is probably the protein that surrounds the fat globules. This suggestion did

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<sup>1</sup> Submitted by M. S. El-Rafey in partial fulfillment of the requirements for the degree, Doctor of Philosophy, University of California, May, 1941.

not clarify the question to any extent. The protein of the stabilizing film on fat globules in milk has been identified as (a) albumin, (b) glycoprotein, (c) globulin-like protein, (d) new protein, (e) casein and (f) a mixture of proteins (6).

Hekma and Brower (15) concluded from microscopical studies that the separator slime came from collapsed foam cells. Grimmer and Schwartz (13) studied the nature of the foaming material in milk by analyzing the separator slime, and found that it contained only 36 per cent casein in the protein fraction. The other 64 per cent protein was claimed to be a new protein probably identical with the foaming substance of milk. Rahn and Sharp (32) called this substance "Schaumstoff."

Ansbacher, Flanigan and Supplee (1) eluted a protein fraction from high-foaming commercial casein. The eluted casein lost its foaming ability, whereas the elution product showed remarkable foaming. There was no definite conclusion as to the identity of this foaming substance, but a globulin with a sterol prosthetic group was suggested. According to the authors, the foaming compound can be separated from milk after removing all the major proteins of milk.

Sharp, Myers, and Guthrie (38) analyzed separated milk foam for the protein fractions. They concluded that there is no preferential accumulation of any major protein fraction in the foam. Sharp and Krukovsky (37) reported the isolation of an agglutinin from milk, which is responsible for the clustering of solid fat globules and the creaming of milk, and also for the foaming of skim milk separated at 50° C.

The modern conception of the factors and forces governing different stages in the existence of a foam will not be reviewed because they have recently been reviewed by Berkman and Egloff (3, 4).

The present investigation represents an attempt to establish a better understanding of the rôle of milk proteins involved in the foaming of milk and some of its products. It includes the isolation and study of the foaming properties of casein, lactalbumin and lactoglobulin.

#### EXPERIMENTAL

*The foam apparatus.* The foam measuring apparatus used in the present work is shown in figure 1. It is a modification of the type used by Hansley (14) and others (5, 8, 12, 18). The chief improvement is the provision for separating the foam from the liquid. A known volume of air is forced at a measured pressure through a sintered glass disc into the layer of liquid above it. The volume and the stability of foam which is formed are measured at the desired temperature.

*Foam measurements.* In standardizing the apparatus, the most reproducible results were obtained with separated milk under the following conditions: 1) volume of sample, 50 ml., 2) time of forcing air through the



sample, 20 seconds, 3) manometer pressure, 30 mm. of mercury and 4) reserve pressure on storage tank, 5 pounds per square inch (0.35 kg./cm.<sup>2</sup>).

The volume of air that passes through the sample varies linearly with the temperature from 195 cc. at 5° C. to 270 cc. at 55° C. with a range of  $\pm 2$  per cent.

To determine the comparative quality of a foam, the sample was tempered in the water bath at the desired temperature and two measurements were obtained. 1) The foam height which is the reading taken immediately after shutting off the air pressure. 2) The average duration of half the

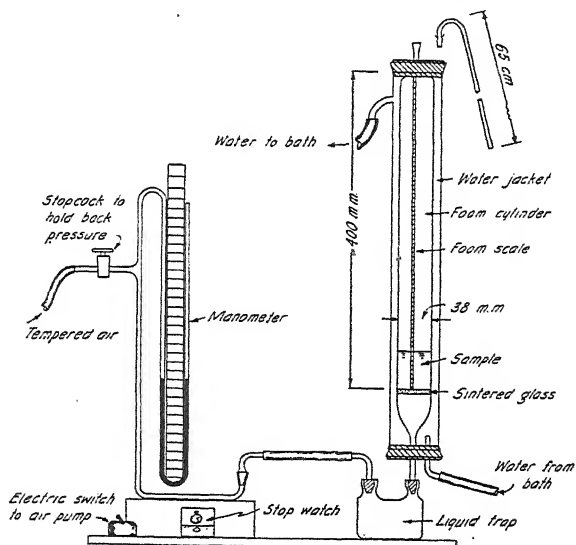


FIG. 1. Foam apparatus. The apparatus consists of a glass cylinder tapered at the lower end to form a ground glass joint. A sintered glass plate is sealed into the tube above the tapered portion. The upper end is sealed to a ground glass joint which permits a connection of a bent glass tube. The cylinder is surrounded by a removable water jacket connected to a pump that circulates the water from a thermostatically controlled water bath. The air pressure is generated by an air compressor provided with a storage tank, a gauge to indicate air storage pressure and a needle valve to control air volume and hence the working air pressure. The temperature and humidity of the air are controlled. A capillary tube mercury manometer connected to the air circuit indicates the pressure to the disc.

volume of the original foam produced, expressed in seconds. The latter is a measure of the foam stability and is termed "half-volume time."

The half-volume time: The introduction of the half-volume time unit proved to be of value in the present study where the stability of a static foam is to be compared with that of dynamic foam, and where the foams do not obey the same subsidence equation. The mathematical equations to calculate the foam life proposed by Lederer (18), Bikerman (5) and Ross and

Clark (8, 35) are not applicable to milk at all temperatures. Figure 2 shows the rate of collapse of raw separated milk foam (0.03 per cent fat) at different temperatures (the time factor in this figure is on a logarithmic scale). It is quite evident that no one formula can be used to calculate the average life of a bubble at all these temperatures. There is an apparent rest period for the separated milk foam before it starts to break down—after which the foam may either gradually subside or explode partially or completely. The “half-volume time”<sup>2</sup> was found to be the best measurement for comparing these foams. It has the advantage over reading the foam height after a certain time interval, which has been used in research on milk foams (19, 22, 36) in that it shows the relative stability of foam that sub-

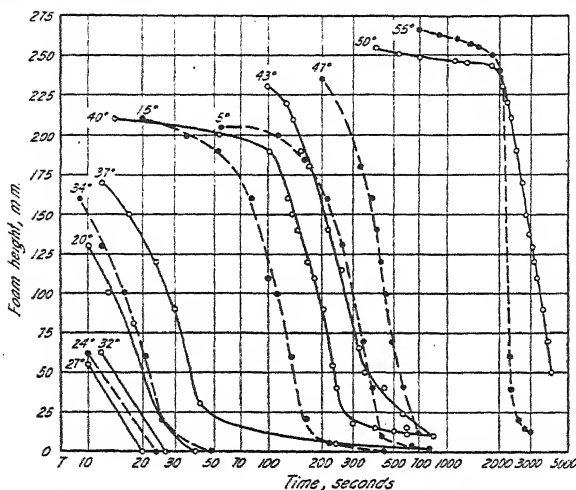


Fig. 2. The effect of temperature on the rate of collapse of raw skim (0.03 per cent fat) foam.

sides completely before the time interval or remains unchanged after the chosen time. This measurement, however, has the disadvantage of being time-consuming and requires constant observation of the foam until it breaks to half its volume. In order to minimize this disadvantage and still get comparable results, an upper limit for the stability was set. This limit was selected as 3,000 seconds, which is approximately the highest value obtained for milk and separated milk foams within the limits of temperature (5 to 55° C.) chosen in this study. The half-volume time of the samples studied at different temperatures seems to be independent of the foam height. This unit can be readily converted to  $\Sigma$ , the average life of a foam bubble, in case of logarithmic subsidence, using the equation  $\Sigma = \frac{\text{half-volume time}}{2.303 \log 2}$ .

<sup>2</sup> After completion of this work, a somewhat similar unit of measuring the foam stability was used by F. Schultz (Trans. Faraday Soc. 38: 85-93, 1942) which is called “foam-time.” An excellent account of this unit of foaminess is presented in his paper.

*Other measurements.* The surface tension measurements were made by a Cenco-du-Noüy interfacial tensiometer. The readings were made within one minute after the surface had been renewed by stirring. Viscosity determinations were made by the Ostwald viscosimeter. For the determination of nitrogen a semi-micro Kjeldahl distillation apparatus, described by Redemann (33) was used, and 30 per cent hydrogen peroxide was added to aid the final oxidation. The pH values were checked with a quinhydrone electrode using a saturated calomel cell and a gold electrode.

#### ISOLATION OF MILK PROTEINS

*Casein.* A survey of the literature (9, 16, 34, 43, 44, 46, 47) shows that there is no method available to separate casein from milk which would be suitable for physico-chemical studies, if the results are to be interpreted in terms of the casein as it exists in milk. Several attempts were made to devise a procedure for isolating casein which is very low in fat and in a non-denatured form. The following was found to be a very suitable procedure: Raw skim milk, separated at 32° C., cooled to room temperature, was passed twice through a Sharples supercentrifuge at 36,000 rpm. The milk fat content of the skim milk as determined by the Babcock method was thus reduced from 0.015 per cent to 0.01 per cent to 0.00 per cent successively. The protein concentration decreased from 3.3 per cent to 2.8 per cent. The supercentrifuged skim milk was held overnight at 2° C. and the casein was precipitated by adding 0.1 N HCl according to the recommendations of Van Slyke and Baker (47). After complete sedimentation of the casein, the whey was filtered off by suction in a large Buchner funnel over filter paper. The casein then was washed thoroughly twice with cold distilled water acidified with HCl to pH 4.6, and three more times with cold distilled water. The casein was filtered by suction after each washing, then the moist casein was spread out in a glass dish and placed in the ice room at -10° C. overnight. The frozen casein was then dehydrated with the aid of an electric fan. After three successive freezings and dehydrations, the semi-dry casein contained about 41.5 per cent total solids and 40.2 per cent protein. The fat content as determined by the Mojonnier method was 0.09 per cent calculated on the dry basis. The casein obtained was in the form of white fluffy particles which were easily soluble in calcium hydroxide and had a very high foaming capacity.

Calcium caseinate solutions were prepared according to Palmer's method (27) with a slight modification. The casein was ground with sufficient calcium hydroxide powder to bring the solution to pH 6.6 (instead of pH 8.0 and subsequent neutralization to pH 6.6 with phosphoric acid). The concentrated casein solutions (about 3.3 per cent) thus obtained were diluted with distilled water and phosphate buffer solution (Sorensen) to give the desired concentration.

*Lactoglobulin.* The lactoglobulin fraction in separated milk is about 1.5 grams per liter. It is obviously desirable, therefore, to employ some simple means of concentrating the whey before submitting it to procedures involving salting out and dialysis. Palmer and Richardson (28) and Palmer (26), described a simple and safe method by which the whey was concentrated by freezing. The concentrated whey (3:1) thus obtained was half-saturated with 18 grams of anhydrous sodium sulfate per 100 ml. of whey and left for eight hours to precipitate at room temperature (the brownish white precipitate concentrated at the surface of the container). The lactoglobulin was filtered, washed with half-saturated solution of sodium sulfate, dissolved in distilled water, dialyzed against ice water and then against 3 per cent sodium chloride. The solution further concentrated by pervaporation (17), was stored in sterilized bottles at 2° C. and was used within 10 days of its preparation. The interference of the fat with the foaming of lactoglobulin led to the use of supercentrifuged rennet whey or the acid whey obtained from supercentrifuged skim milk. It was found necessary also in some instances to extract the concentrated lactoglobulin solution with hexane.

*Lactalbumin.* The whey filtrate, after removing the globulin fraction, was warmed to 35° C. and sodium sulfate added to bring its final concentration up to 36 grams salt per 100 ml. (26). The white precipitate filtered off at 35° C. was dissolved in distilled water and dialyzed against running water at 2° C. until free from sulfate ion. The crystal clear lactalbumin solution was further concentrated by pervaporation and stored in a sterilized bottle at 2° C. for use within 10 days of its preparation. According to reports by Pedersen (29), one can assume that the lactalbumin thus obtained was composed of the  $\alpha$  and  $\beta$  fractions.

## RESULTS

### *Foaming Properties of Casein*

*Effect of concentration.* The effect of a progressive increase in concentration of casein on the foaming stabilities of its solutions is shown in figure 3. In both the presence (2.7 per cent) and the comparative absence of fat (0.06 per cent dry basis), it is to be observed that with increasing concentration of protein, the stability of the foam increases to a maximum then decreases again. The foam heights (50) on the contrary, were observed to reach a maximum value with no further decrease with increasing concentrations. The surface tension decreases to a constant value. In these experiments, the pH of the solutions was kept at 6.6 by the use of a phosphate buffer. The zone of maximum foam stability seems to be a function of the amount of fat present in the solution and the temperature at which the foaming properties are tested. Low temperature and low fat content favor the stability of calcium caseinate foam.

*Effect of temperature.* Preliminary results indicated that the foam height, foam stability and surface tension of calcium caseinate solutions all decreased progressively as the temperature increased from 5 to 55° C. The greatest effect of temperature was found to be on the stability of the foam. Casein prepared by the Hammarsten or Van Slyke and Baker methods did not give stable foams which might be due to partial denaturation of the protein during the method of preparation. As an example, the half-volume time of the foams of 2.8 per cent calcium caseinate solution (Van Slyke and Baker methods) was 310 seconds at 5° C. and 13 seconds at 55° C. Casein prepared by Cohn and Hendry's method contained 2.7 per cent fat on the dry basis (Mojonnier). The foam stability of this casein was low due to the interference of fat; the half-volume time of 2.4 per cent calcium

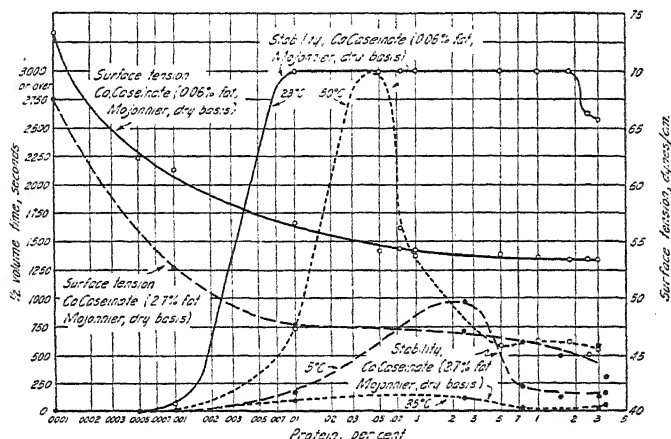


FIG. 3. The effect of concentration on the foam stabilities and surface tension of Ca caseinate solutions.

caseinate solution was about 220 seconds at 5° C. and 17 seconds at 55° C. It was also observed that elution of casein by Ansbacher, Flanigan and Supplee's method (1) did not decrease the foaming capacity or the stability of the calcium caseinate solution. In some cases, an actual increase with the eluted casein was observed which was correlated with a decrease in the fat content of the casein. Figure 4 shows a sample of the results obtained.

Calcium caseinate solutions prepared by the new method described in this paper from ultracentrifuged separated milk yield foams of greater capacity and stability (figure 5). Our data have shown that the surface tension of a 1.1 per cent calcium caseinate solution decreases only slightly with an increase in temperature (from 54.2 dynes at 5° C. to 50 dynes at 55° C.) and the foam heights are approximately proportional to the volume of air introduced at all temperatures (50). The greatest change is observed in the stability measurement, which increases slightly with temperature up to

22° C., then starts to decline slowly at first up to 35° C., and rapidly afterwards to 55° C. This great improvement in the stability of the foam is doubtless related to the lack of denaturation of the protein as well as the low fat content of casein prepared by this method.

*Effect of fat.* The addition of 0.015 per cent milk fat emulsion to the calcium caseinate solution decreases the foam height and the stability of the

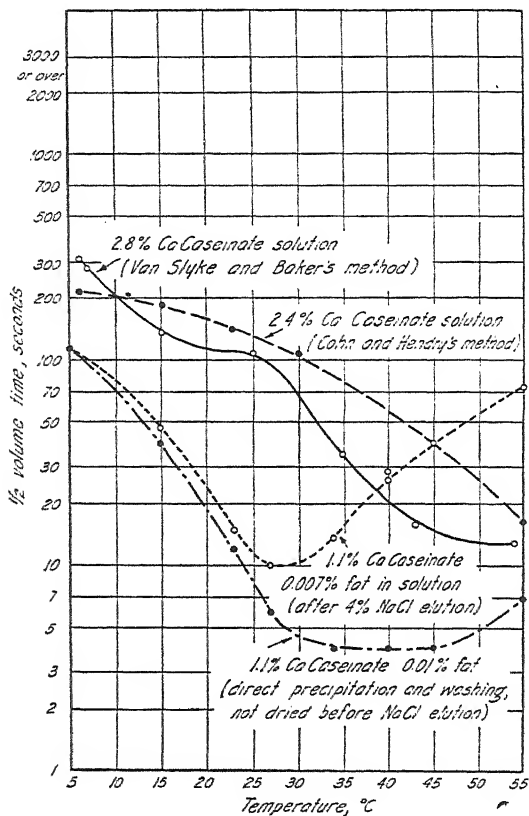


FIG. 4. The effect of methods of preparing casein on the foam stabilities of caseinate solutions.

foam to a marked extent. The reduction of the stability of the foam is greatest at temperatures above 15° C. as shown in figure 5.

The fat emulsion was prepared by emulsifying about 1 per cent milk fat in dilute phosphate buffer at 60° C. followed by cooling in ice water. The emulsion showed marked stability due to the decrease of the fat/solution interfacial tension by the salts.<sup>3</sup>

<sup>3</sup> See Harkins, W. D., Some aspects of surface chemistry fundamental for biology, Jour. Chem. Physics., 6: 171, 1938.

## FOAMING QUALITIES OF LACTOGLOBULIN

*Effect of concentration.* Lactoglobulins prepared from supercentrifuged rennet whey do not give stable foams. The effect of increasing the lactoglobulin concentration from 0.0001 to 0.75 per cent on the foam stability was tested at 25° C. and 50° C. The results plotted in figure 6 indicate that the foaming capacity increases slightly with concentration at 25° C. The results obtained at 50° C. show that there is a subsequent decrease in foam

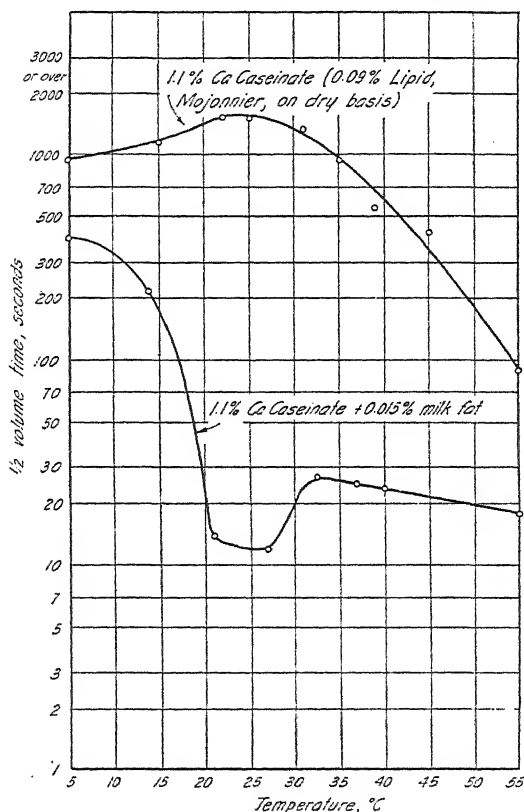


FIG. 5. The foam stabilities of Ca caseinate solutions of casein prepared by the present method and the effect of adding milk fat emulsion.

stability with an increase in concentration. The surface tension continues to decrease as the concentration increases. The foam stability follows the same pattern as that reported for calcium caseinate. On the whole, it is quite clear that lactoglobulin has very poor foaming qualities. This may be attributed to the presence of a lipid material associated with its protein molecule (10, 24).

*Effect of temperature and fat content.* Table 1 shows that 0.05 per cent lactoglobulin solutions (the concentration normally present in milk) do not

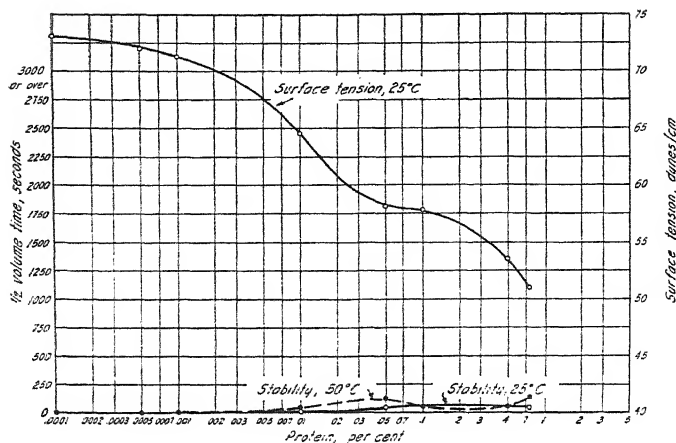


FIG. 6. The effect of concentration on the foam stabilities and surface tension of lactoglobulin solutions.

form stable foams at any temperature tested, when the solution contained about 0.006 per cent fat. As the fat content was decreased to 0.001 per cent by extracting the same solution twice with equal volumes of hexane, the foam height and the stability increase at all temperatures tested, the increase being greater at temperatures above 20° C. Lactoglobulin solutions were prepared in phosphate buffer solutions at pH 6.6.

#### THE FOAMING PROPERTIES OF LACTALBUMIN

*Effect of concentration.* The effect of increasing the concentration of lactalbumin at 25° C. and 50° C. is illustrated in figure 7. As the concentration increases from 0 to 1 per cent the foam stabilities increase to maximum values and remain unchanged (the foam heights were observed also to reach maximum values (50)). The surface tension continues to decrease

TABLE 1

*Foaming properties of lactoglobulin. Effect of temperature on 0.05% lactoglobulin solutions of different fat contents*

0.05% lactoglobulin precipitated once from rennet whey (0.0065% fat in the solution)				Same globulin solution extracted twice with hexane (0.001% fat in the solution)			
Temperature	Surface tension	Foam height	½ volume time	Temperature	Surface tension	Foam height	½ volume time
°C.	dynes	mm.	sec.	°C.	dynes	mm.	sec.
5.0	54.4	45	5	6	49.0	120	20
15.0	51.6	25	5	10	49.1	115	19
22.5	49.4	8	2	17	.....	100	17
32.0	48.2	40	4	20	48.0	120	21
43.0	47.5	190	20	32	49.3	220	50
52.0	47.1	260	72	40	46.9	260	380



to a minimum value. It is of interest to note that lactalbumin is more surface-active with regard to foaming, at 50° C. than at 25° C., as evidenced by the fact that less protein concentration is required to support a stable form at the former temperature.

*Effect of temperature.* The results illustrated in figure 8 show that a 0.047 per cent lactalbumin solution forms a much more stable foam than a corresponding concentration of lactoglobulin solution. The half-volume time increases with the rise of temperature from 5° C. to 23° C. where it reaches the maximum limits set for this study and remains so up to 50° C. When the protein concentration is increased to 0.18 per cent, the foam stability exceeds the limits at all temperatures. The foam volumes were found to be equal to the volume of air forced through the solutions (50).

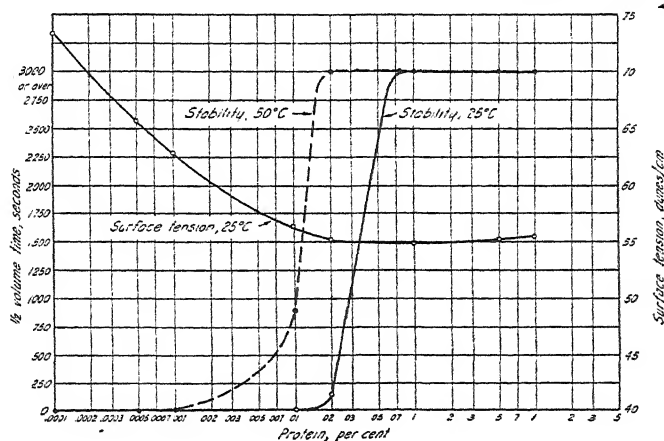


FIG. 7. The effect of concentration on the foam stabilities and surface tension of lactalbumin solutions.

*Effect of fat.* The effect of adding different amounts of milk fat emulsion stabilized in dilute phosphate buffer is quite interesting—figure 8. The addition of 0.0005 per cent fat to a 0.05 per cent lactalbumin solution decreases the stability of the foam greatly at temperatures below 25° C. and to a less extent at 30.5° C. but has no effect above 38° C. When the fat content is further increased to 0.005 per cent in 0.42 per cent lactalbumin solution, the reduction in the foaming properties is much greater at all temperatures. These changes are accompanied by a lower surface tension. A further increase of the fat content to 0.5 per cent in the 0.05 per cent lactalbumin solution, decreases the foam measurements (and surface tension (50)) to still lower limits. It is clear then that the foaming capacity of a lactalbumin solution is a function of the amount of fat present and the temperatures at which the foaming properties are measured. The inhibiting effect of milk fat is reduced at temperatures above its melting-point. The

next experiments were designed to study the effect of increasing the albumin content in a solution, the foaming properties of which were greatly inhibited by the presence of a relatively high fat concentration. As shown in figure 8, increasing the concentration of albumin from 0.05 to 0.5 per cent in a solution containing 0.5 per cent milk fat improves the stability of the foam only slightly at temperatures below 39° C. but to a great extent above that tem-

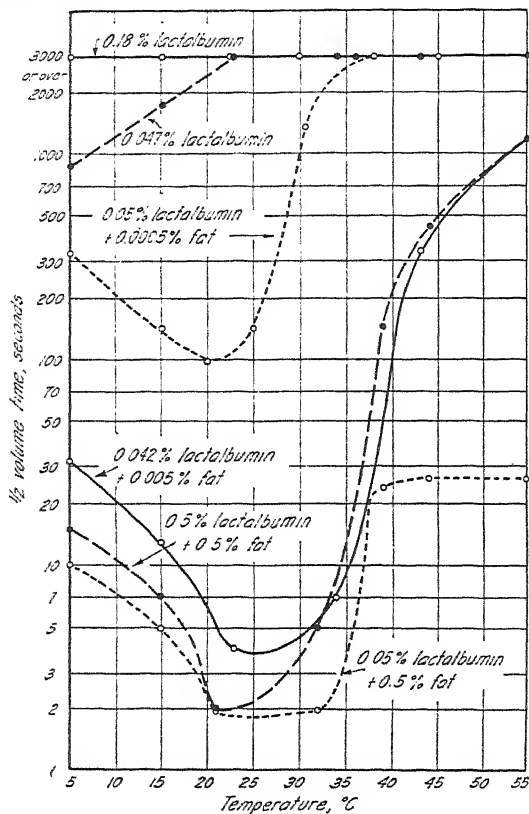


FIG. 8. The foam stabilities of lactalbumin solutions with different amounts of milk fat emulsion added.

perature. The surface tension values are about the same in both solutions (47.3–47.0 dynes respectively (50)).

#### DISCUSSION

The experimental results have shown that the foaming properties of solutions containing surface-active constituents of milk vary markedly with a number of factors depending on the chemical nature and the amount of materials present in the solution, and the physical conditions under which the measurements are obtained. The variations in the foaming properties

of milk proteins can be discussed best if only the stability of the foam is considered, since in some instances the foam heights are equal while the stabilities are quite different.

The effect of increasing the concentration of calcium caseinate or lactoglobulin in buffered solutions at pH 6.6 results in a progressive increase in the foam stability to a maximum followed by a subsequent decrease. The latter decrease is not noticed in the case of lactalbumin solutions up to 1 per cent concentration. The explanation of these facts cannot be based on changes of surface tension<sup>4</sup> or viscosity, a conclusion which has been reached by several investigators (11, 23, 30, 49). The same phenomenon of the relation of concentration to the foam stability, observed by Bartsch (2) on simple organic compounds, was explained on the boundary heterogeneity theory. Thus, the foam stability increases when the air/solution interface becomes heterogeneous with respect to the solvent and the solute (a condition which is not present in very dilute or concentrated solutions). Talmud (45) relates the maximum foam stability to the concentration that allows for maximal hydration at the interface. At the optimum concentration the organic molecules are oriented, inclining at a certain angle to the surface and interlacing with each other to form a kind of gel structure or a film with the highest tensile strength. Therefore, in order to discuss the stability of protein foams, consideration should be given to the micellar size, solubility, orientation, and the degree of hydration, when such information is available. Lactalbumin, being highly soluble in water and having a low molecular weight (17,500–39,000) (29) might well be placed with the soluble compounds in Bartsch's classification of the foaming substances (2). On the other hand, casein and lactoglobulin with a low solubility in water and with high micellar weights (average 87,000 and 140,000 respectively) (29) may be placed with insoluble substances in the same classification.

The effect of temperature on the foam stability of milk protein solutions may be explained on the same principle; *i.e.*, there is an optimum molecular size and degree of hydration that permits the formation of a lamella which has a high tensile strength. For example, a solution of calcium caseinate (figure 5) forms a foam that has a half-volume time of approximately 1520 seconds at 22° C. and 90 seconds at 55° C. This rapid decrease in foam stability at temperatures above 35° C. can be correlated with the fact observed by Svedberg, Carpenter and Carpenter (44) that the micellar size of calcium caseinate is doubled by warming its solution to 40° C. These authors believe that the heating causes a polymerization or association of the molecules to form larger aggregates. Such an increase in size along with the dehydration of casein upon heating (23, 48) would seem to be among the important

<sup>4</sup> K. G. A. Parkhurst, in a recent paper (Trans. Faraday Soc., 37: 496–505, 1941), stated that it is not low surface tension *per se* that ensures foam stability but the nature of the adsorbed layer giving rise to the lowering of surface tension.

factors responsible for the decrease of foam stability of a calcium caseinate solution heated to 55° C. Further evidence on the importance of the particle size of calcium caseinate to the foam stability of its solution can be obtained from correlating the findings of Carpenter (7) with the foam stability obtained in this study. According to Carpenter, the molecular weight of casein in a 1.5 per cent solution is 96,000 at pH 6.8 (M/60 phosphate buffer), but on dilution to less than 0.7 per cent, the micelle dissociates to one-third the initial weight, namely 32,000. This dissociation is completely reversible and thus confirms Sorenson's conception (41, 42) of a protein as a complex undergoing reversible association and dissociation. This decrease in micellar size on dilution is accompanied by changes in physical properties, as for example, an increase in specific rotation. The foam stability of different concentrations shown in figure 3 indicates that as the micellar weight of calcium caseinate decreases by dilution, the foam stability increases. For example, at 50° C. the half-volume times are 630 and 1370 seconds, corresponding to concentrations of 1.0 and 0.1 per cent respectively. At a concentration of 0.05 per cent, a calcium caseinate solution (prepared from unaltered casein) gives an apparently clear solution that has about the same micellar size (7) and foam stability as a 0.05 per cent lactalbumin solution. Likewise, sodium caseinate appears to have a smaller micellar size than calcium caseinate (43b) and the results, not reported in this paper, show that it has higher foam stability.

It is quite important to note that none of the casein solutions, at concentrations above 0.5 per cent, supports a stable foam at temperatures above 43° C., where the skim milk forms a very stable foam.

No data are available on the changes of particle weight or the degree of hydration of lactalbumin or lactoglobulin as the temperature is increased from 5 to 55° C. The facts reported in table 1 and figure 8 indicate that both protein solutions have greater stability at higher temperatures and, by analogy, it may be reasoned that the conditions favorable for forming lamellae with higher tensile strength and greater stability are better fulfilled at these temperatures. One of the present trends of thought in protein chemistry (21) suggests that the larger protein molecules consist of small primary protein units linked into a secondary structure by means of carbohydrate, phospholipid, nucleic acid or polyvalent inorganic ions, and that these units are subject to association and dissociation under different conditions (7, 21, 41, 42). It may be of value to consider the foaming stability as one of the physical properties that reflects such an association and dissociation as pointed out in discussing the foaming properties of calcium caseinate solutions.

The effect of adding small amounts of milk fat emulsion in phosphate buffer to milk protein solutions is quite interesting. The data indicate that the foam stabilities of lactalbumin solutions are reduced to a greater extent

by the fat at temperatures below the melting point of the milk fat (figure 8). The reverse is observed in calcium caseinate solutions (figure 5). A 0.05 per cent lactoglobulin solution does not give a stable foam in the presence of as little as 0.0065 per cent fat, yet the stability of the foam is greater at temperatures beyond the melting point of milk fat (table 1). The action of milk fat on the foaming properties will be discussed in a later paper.

#### SUMMARY

An apparatus is described for accurate measurements of the foaming properties of milk. Experimental data have revealed that the subsidence of skim milk foams cannot be defined by a single equation at temperatures between 5 and 55° C. For this reason, a unit called a "half-volume time" has been proposed and used to compare the stabilities of static and dynamic foams. The unit can be converted by a given equation to give average life of a foam bubble, if the foam follows a logarithmic subsidence.

The major surface-active proteins of milk, including casein,  $\alpha$ - $\beta$ -lactalbumin and lactoglobulin, have been separated, purified and studied for their foaming properties. A new procedure for isolating undenatured casein with a lipid content as low as 0.06 per cent has been introduced. The method is based on super-centrifuging skim milk, precipitating the casein at a low temperature with 0.1 N HCl, washing, freezing, and dehydrating the frozen casein by pervaporation.

The foam "half-volume time" values of milk protein solutions in M/30 phosphate buffers at the normal hydrogen ion concentration of milk varied between 2 and 3000 seconds (or over), depending upon the kind of protein, concentration, method of isolating the protein, temperatures, and the amount of milk lipids present in association with the protein molecule or added in the form of an emulsion to the solution. Studies of these variables are reported and some theoretical explanations advanced.

It is shown that calcium caseinate solutions of a concentration equivalent to that of milk and prepared by the present procedure have high foam stabilities at temperatures below 40° C. Lactalbumin solutions foam well at all temperatures, but lactoglobulin solutions show no appreciable foaming. The foam depressing action of milk fat is shown to be greater at temperatures over 15° C. with calcium caseinate solutions and at temperatures lower than 35° C. with lactalbumin solutions.

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# THE ROLE OF SURFACE-ACTIVE CONSTITUENTS INVOLVED IN THE FOAMING OF MILK AND CERTAIN MILK PRODUCTS.

## II. WHEY, SKIMMED MILK, AND THEIR COUNTERPARTS

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### REVIEW OF LITERATURE

The foaming properties of some milk products represent a unique colloidal phenomenon; that is, the foam stability decreases to a minimum, as the temperature increases, after which it again increases. The first report on the effect of temperature was made by Siedel (22). He found that the quantity of foam depends on the temperature of the milk and that the nature of the foam at low temperature is different from that at high temperature. Siedel's studies did not go beyond 35° C., but he noticed an inflection in the stability of the foam. Leete (11) and Sanmann and Ruehe (18) studied the effect of temperature on milk foam. Separated milk and milk foamed least between 20 and 30° C. according to the former, and at 27° C. according to the latter. Both investigators agreed on the increase in the foam at temperatures beyond 30° C. but disagreed on the temperature at which the maximum foaming appeared.

There has been considerable speculation as to the cause of this minimum foaming ability of milk. Rahn and Sharp (15) raised the question whether these kinks in the foam-temperature curve indicate the presence of a specific foam-producing material or of a stabilizing substance in the foam. Ansbacher and co-workers (1) reported that the foam-producing substance eluted from casein showed a critical agglomeration at the temperature of the minimum foaming. They did not present data regarding the foaming stability of this highly foaming constituent of milk. Leviton and Leighton (12) tried to explain this kink on the basis of the work of King (10), who showed that the portion of the surface of milk covered with a film of fat increased with a rise in temperature from 10 to 20° C. They postulated that the decrease in the foaming power might be explained on the basis of the increase in the tendency of the fat to spread as the temperature rose. No comment was made on the further increase of foam formation with increasing temperature.

Holm (9) explained the minimum foaming of milk as a result of variation in those properties of the solution which evidence themselves in viscosity

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changes. Davies (3) considered that the range from 20 to 30° C. represents conditions in which hydration is practically nil and the stabilizing factors, *i.e.*, the decrease in surface tension and denaturation of the proteins, have not come into play. Ansbacher *et al.* (1), however, found that the viscosity of their foam material was the same as that of water.

The authors are not aware of any published work on the foaming properties of whey.

In a previous paper (5a) the authors reviewed the existing controversy as to the foaming constituents in milk. Using the "half-volume time" for measuring the foam stabilities of the major milk proteins, it was found that calcium caseinate solutions have high foam stabilities at certain concentrations at temperatures below 40° C.; lactalbumin solutions foam well at all temperatures, but lactoglobulin solutions show no appreciable foaming properties. Foam-depressing action of milk fat was found to be greater at higher temperatures with calcium caseinate solutions, and at lower temperatures with lactalbumin solutions.

The present work deals with the preparation of synthetic whey and separated milk by combining the milk protein fractions in an attempt to duplicate the foaming properties of these products. As a result an explanation of the effect of heat on the foaming properties of whey, separated milk and milk is offered.

#### EXPERIMENTAL

The foam measuring apparatus, technique, and other methods used have been described in a previous paper (5a). Separated milk and rennet whey used in this work were obtained from the University Farm Creamery. The foaming properties of the solutions studied were compared at different temperatures between 5 and 55° C.

*The foaming properties of whey and synthetic whey.* The variation of the foam stabilities of various wheys with temperature is shown in figure 1. As the temperature increases from 5 to 55° C. the foam stability of the ordinary rennet whey (0.035 per cent fat) decreases to a minimum value between 22 and 32° C. and is followed by an enormous increase at higher temperatures. On combining solutions of lactalbumin and lactoglobulin separated from this whey, to give concentrations of 0.61 per cent and 0.05 per cent respectively in a phosphate buffer of the same pH as the whey, the foaming properties were found to be practically the same as the original whey. The type and general characteristics of the foam at low temperatures were identical with those of lactoglobulin solutions; those at temperatures above 32° C. resembled lactalbumin solutions.

In order to reduce the fat content of the whey and of the lactalbumin and lactoglobulin separated from it without the use of fat solvents the whey was supercentrifuged. The fat content of the whey was thereby decreased from 0.04 per cent to about 0.0001 per cent, and the protein decreased by

0.02 per cent. The foaming properties were greatly improved. A similar improvement in the foam height and stability were obtained with acid whey prepared from supercentrifuged skim milk (5b).

Solutions of lactalbumin and lactoglobulin (prepared from supercentrifuged whey), when combined to give the same concentration as in whey, give essentially the same foaming properties as the supercentrifuged whey itself (figure 1).

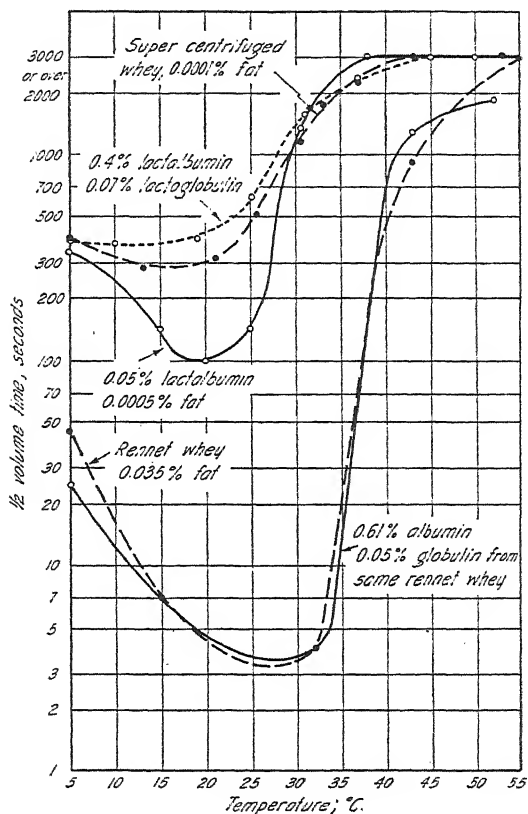


FIG. 1. The foam stabilities of whey with different fat concentrations and their duplication with lactalbumin solutions with fat or lactoglobulin added.

In a former paper (5a) it was shown that lactalbumin solutions (0.10 per cent or over) give stable foams represented by half-volume times greater than 3000 seconds at all temperatures. The present data indicate that the presence of lactoglobulin (from supercentrifuged whey) in a 0.4 per cent lactalbumin solution reduces the foam stability of the latter at lower temperatures to about 10 per cent of its original value. The addition of a milk fat emulsion in phosphate buffer to a lactalbumin solution (ratio 1:100 in solution), however, is shown to have an effect similar to the lactoglobulin.

On the other hand, when lactoglobulin, separated from ordinary rennet whey (0.035 per cent fat) is added to a 0.61 per cent lactalbumin solution, the foam stability of the latter is greatly reduced at the lower temperatures. The mixture has about the same foam stability as the original whey. The further reduction in this case undoubtedly is due to the presence of fat in the isolated lactoglobulin.

*The foaming properties of separated milk.* Raw separated milk (0.01 per cent fat by Babcock test) obtained from mixed herd milk forms a rela-

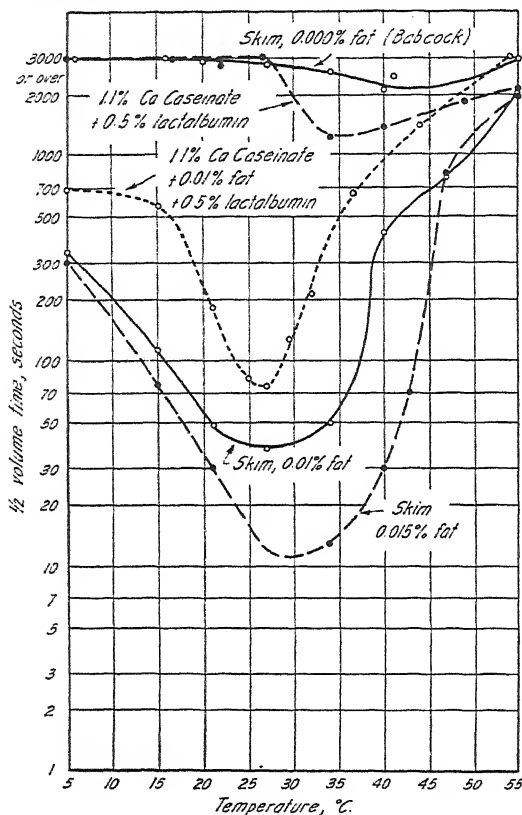


FIG. 2. The foam stabilities of skim milks with different fat content and their duplication with Ca caseinate + lactalbumin with and without milk fat added.

tively high stable foam at 5° C. On increasing the temperature to 25° C., both the foaming capacity and the half-volume time of the foam are reduced from 200 mm. and 429 seconds to 170 mm. and 21 seconds respectively. By increasing the temperature to 55° C. the foaming properties are improved again until a second maximum, much higher than the first, is reached (figure 2). The type of the foam changes also with the increase in temperature; that is, from relatively large and loose, to small, compact foam cells.

The effect of decreasing the fat content by passing the separated milk through a Sharples Supercentrifuge (36,000 rpm.) is shown in figure 2. As the fat content decreased from 0.015 to practically zero per cent (Babcock test), the skim milk showed remarkable foaming properties at all temperatures. The half-volume time of the foam at 27° C. increased from about 37 seconds (original) to about 2800 seconds (super-skimmed). There is no minimum foaming of supercentrifuged skim milk, which indicates that the

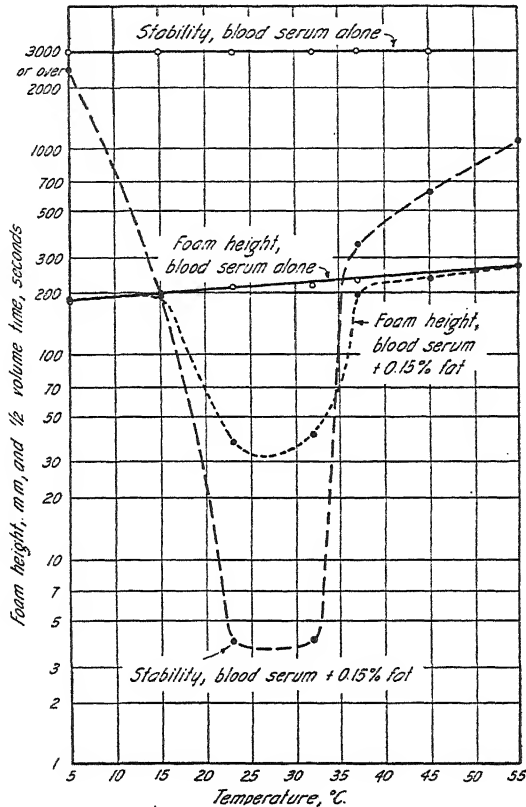


FIG. 3. The effect of adding fat emulsion on the foaming properties of "horse blood serum."

tendency of ordinary separator milk to give minimum foam values at room temperature is due to the presence of the fat globules.

*Effect of adding fat emulsion to blood serum.* To confirm further that the minimum foaming of separated milk is mainly due to the presence of fat globules, horse blood serum, obtained by defibrinating fresh blood with glass beads, was tested for foaming properties before and after the addition of a 0.15 per cent milk fat emulsion in phosphate buffer. The results reported in figure 3 show that the "half-volume time" is reduced by the presence

of fat from over 3,000 to 4 seconds at room temperature. Further increases or decreases in temperature improve both the foaming capacity and stability.

*Comparison of the separated milk and whey foams.* In comparing the foam-temperature curves of the separated milk (figure 2) and whey (figure 1) in the presence and absence of fat, it becomes evident that skim milk has a relatively higher foam stability in the temperature range from 5 to 35° C. than the corresponding whey. The main difference in the chemical composition of the skim milk as compared with the whey lies in the absence of casein from the latter. Therefore, it is reasonable to assume that the presence of calcium caseinate accounts for the greater stability of skim milk foam at lower temperatures.

*Foaming of a reconstructed separated milk.* On the basis of the previous findings that the constituents responsible for the foaming of the whey are lactalbumin and a lipid fraction, it was anticipated that by adding non-

TABLE 1

*The effect of the addition of 0.05 per cent lactoglobulin\* on the foaming properties of a 1.1 per cent calcium caseinate (0.09 per cent fat, dry basis) solution*

Temperature	Surface tension		Foam height		Half-volume time	
	Calcium caseinate	Ca caseinate plus lactoglobulin	Calcium caseinate	Ca caseinate plus lactoglobulin	Calcium caseinate	Ca caseinate plus lactoglobulin
°C.	<i>dynes/cm.</i>	<i>dynes/cm.</i>	<i>mm.</i>	<i>mm.</i>	<i>sec.</i>	<i>sec.</i>
5	54.2	54.6	201	200	935	850
15	53.7	52.8	213	215	1170	1030
25	51.0	51.6	220	218	1500	1525
35	51.0	50.6	247	241	950	595
45	50.2	49.4	245	235	423	182

\* Prepared from twice supercentrifuged skim milk.

denatured calcium caseinate to these two constituents a solution would be obtained having the foaming properties of skim milk. The results obtained and shown in figure 2 confirmed that idea. It is possible to get an approximate duplication of the foaming characteristics of skim milk (0.01 per cent fat) with a solution containing 1.1 per cent calcium caseinate, 0.5 per cent lactalbumin, and 0.01 per cent milk fat emulsion in phosphate buffer. The increase observed in the foam stability of the synthesized solution can be accounted for by the absence of the phospholipid fraction which normally surrounds the fat globules. The effect of the fat globule membrane in determining the depressing action of the fat on the foam was previously studied (5). A buffered solution (pH 6.6) of 1.1 per cent calcium caseinate and 0.5 per cent lactalbumin gives approximately the same foam stabilities as the supercentrifuged skim milk (0.00 per cent fat by the Babcock test). Both the reconstituted and the supercentrifuged skim milk form foams of remarkable stability at all temperatures. The "half-volume times" of the foams

Table 1 shows that the addition of 0.05 per cent lactoglobulin to a calcium caseinate solution does not change the foaming properties of calcium caseinate appreciably except at the higher temperatures at which the fat is liquid. The lower foam stability of the mixture at 35° and 45° C. is to be expected in view of the additional lipid material contributed by the lactoglobulin.

From these results it is concluded that the foaming properties of skim milk are due to the presence of calcium caseinate, lactalbumin and milk fat. A solution containing 0.6 per cent lactalbumin, 0.05 per cent lactoglobulin, and 2.7 per cent calcium caseinate (which is prepared from casein extracted with hot alcohol, ether and dried) however, does not have the same foaming properties as skim milk (Table 2). The results support the belief that the physical properties of the casein treated with alcohol and ether are altered.

TABLE 2

*Foaming properties of synthetic solutions containing casein which has been treated with alcohol and ether, in comparison to skim milk*

No.	Sample	30° C.		43° C.		55° C.	
		Foam height	$\frac{1}{2}$ volume time	Foam height	$\frac{1}{2}$ volume time	Foam height	$\frac{1}{2}$ volume time
		<i>mm.</i>	<i>sec.</i>	<i>mm.</i>	<i>sec.</i>	<i>mm.</i>	<i>sec.</i>
1.	2.7% Ca caseinate solution (casein extracted with alcohol and ether and dried)	182	49	190	16	160	13
2.	2.8% Ca caseinate from casein No. 1, plus 0.6% lactalbumin and 0.05% lactoglobulin in solution	140	18	150	17	205	54
3.	Skim milk + 0.018% fat	170	22	240	400	260	2005

*The relation of "agglutinin" to the foaming of separated milk.* Sharp and Krukovsky (19) attributed the high foaming properties of skim milk separated at 50° C. to the presence of an agglutinin and the poor foaming of that separated at 5° C. to the absence of agglutinin. This work is not in agreement with the present findings that calcium caseinate and lactalbumin are the chief foaming proteins in skim milk. Consequently, their work was repeated with the hope of finding the reason for such a difference in the foaming of separated milk obtained at 5° C. and 50° C.

Preliminary results obtained by separating raw milk at 5° C. and 32° C. indicated that the skim milk samples separated at 5° C. behaved abnormally with regard to the foaming properties; that is, the stability of the foam was reduced at 5° C. and at 55° C., but was increased at room temperature. Further research revealed that the resulting complications arose from two factors: 1) a slight development of rancidity which is probably due to centri-

TABLE 3

*Effect of the temperature of separation on the foaming ability of the recombined milks*

4.25 per cent pasteurized milk separated at two temperatures—at 5° C. (skim milk 0.11 per cent fat and 22.6 per cent cream) and at 53° C. (skim milk .015 per cent fat and 23.5 per cent cream). All the possible combinations of 4.25 per cent milks were prepared—the foaming properties of the original raw milk and the skim milks were tested at 3 different temperatures.

Sample	5° C.			25° C.			50° C.		
	Surface tension <i>dynes/cm.</i>	Foam height <i>mm.</i>	$\frac{1}{2}$ volume time <i>sec.</i>	Surface tension <i>dynes/cm.</i>	Foam height <i>mm.</i>	$\frac{1}{2}$ volume time <i>sec.</i>	Surface tension <i>dynes/cm.</i>	Foam height <i>mm.</i>	$\frac{1}{2}$ volume time <i>sec.</i>
(1) Raw milk .....	53.0	204	47	46.9	35	5	.....	270	1800
(2) Pasteurized milk .....	53.6	200	34	47.3	22	4	.....	288	3000
(3) 53° C. skim 0.015% fat .....	54.9	200	460	53.5	180	19	.....	280	3000
(4) 5° C. skim 0.11% fat .....	54.8	200	128	48.9	40	4	49.9	275	3000
(5) 53° C. skim 0.11% fat .....	55.0	202	133	48.6	20	3	46.1	276	3000
(6) 53° C. skim + 53° C. cream .....	53.5	198	27	47.5	30	4	.....	287	3000
(7) 5° C. skim + 5° C. cream .....	53.5	200	30	47.4	26	4	.....	284	3000
(8) 53° C. skim + 5° C. cream .....	53.6	200	30	47.8	23	4	.....	286	3000
(9) 5° C. skim + 53° C. cream .....	53.2	197	29	47.6	26	4	.....	284	3000

Cream Volume Measurement

Sample	Cream volume %				
	Hours				
	0	2 $\frac{1}{2}$	6 $\frac{1}{2}$	8 $\frac{1}{2}$	20
(2) Pasteurized milk .....	0	17.0	16.5	16.0	15
(6) 53° C. skim + 53° C. cream .....	0	15.0	14.75	14.75	14
(7) 53° 7. skim + 5° C. cream .....	0	15.0	15.0	14.5	14
(8) 53° C. skim + 5° C. cream .....	0	18.0	17.0	16.5	15
(9) 5° C. skim + 53° C. cream .....	0	3.5	6.5	8.0	9



fuging milk at low temperatures:<sup>2</sup> 2) the higher fat content in the skim milk due to inefficient separation at low temperatures.

The data on the effect of rancidity and homogenization on milk foam will be reported in a later paper. Our results show that a slight development of rancidity results in a marked decrease in the stability of milk foam at 5° and 50° C., while it increases the foam stability at temperatures between 10 and 30° C.

According to these facts, the assumption was made that if the work of Sharp and Krukovsky were to be repeated with pasteurized milk (61.5° C. for 30 minutes) and the fat were to be increased to correspond to that separated at low temperature, then both skim milks would have the same foaming properties. The assumption was borne out by the experimental results reported in table 3.

These facts do not exclude the possibility of the presence of an agglutinin in milk because the measurements obtained on the cream volume of reconstituted milks agree with the results of Sharp and Krukovsky. However, the data indicate clearly that the agglutinin is not one of the constituents which determine the foaming properties of milk.

#### DISCUSSION

The data presented in figure 1 show the similarity in the foaming properties of whey and synthetic solutions containing lactalbumin and lactoglobulin in their normal concentration. They show also that the addition of small amounts of milk fat to lactalbumin solutions gives foaming properties similar to whey. In other words, the reduction in the foam stability of lactalbumin solutions that results from adding lactoglobulin is probably due to the presence of small amounts of fat associated with lactoglobulin. Therefore, it is reasonable to conclude that lactalbumin and milk fat are the major constituents that determine the foaming properties of whey.

In figure 2 it is shown that the minimum foaming of separated milk at room temperature is due to the presence of fat in the separated milk. When the fat globules were largely removed by passing that separated milk through a supercentrifuge, it showed about the same foaming properties at all temperatures tested. It has been shown also that the essential foaming characteristics of supercentrifuged skim milk can be duplicated with a solution of calcium caseinate plus lactalbumin, and that the addition of 0.01 per cent milk fat to this solution decreases its foam stability to a minimum at 27° C. Similar observations on horse blood serum are reported in figure 3. These results are taken to indicate that the presence of fat globules in biological

<sup>2</sup> Sharp and Tomasi (21), and Doan (4) have also reported on the development of rancidity in samples separated at low temperatures and pointed out that the action of centrifugal separation is somewhat similar to homogenization in that it stimulates hydrolysis of the fat in the resulting cream.

fluids reduces their foaming properties to a great extent at certain temperatures, a fact which may prove of value in testing for lipemia.

To explain the foaming properties of skim milk or milk, we are led to the belief that calcium caseinate is the foaming substance of milk at low temperatures, while lactalbumin is the substance contributing to the foam at temperatures above the melting point of milk fat. This conclusion is supported by the experimental data presented and deduced from the following considerations.

(a) A calcium caseinate solution does not form a stable foam at temperatures above 21° C. in the presence of 0.015 per cent fat (5a). At 50° C. the "half-volume time" of such a solution is of the order of 20 seconds, while that of the ordinary skim milk or milk is over 1,000 seconds.

(b) A lactalbumin solution in the presence of 0.005 per cent fat does not form a stable foam at temperatures below 34° C. but forms a stable foam at higher temperatures corresponding to those of milk and of skim milk (5a).

TABLE 4

No. of times through separator	Sample	Protein			
		Total	Casein	Heat coagulable	Non-heat coagulable
		%	%	%	%
2	Original milk	3.89	3.05	0.40	0.44
	Foam	4.19	3.35	0.40	0.44
	Difference	0.30	0.30	0.0	0.0
5	Original milk	3.69	2.89	0.37	0.43
	Foam	4.00	3.20	0.35	0.45
	Difference	0.31	0.31	- 0.02	+ 0.02
8	Original milk	3.57	2.77	0.37	0.43
	Foam	3.82	3.00	0.39	0.43
	Difference	0.25	0.23	0.02	0.0

(c) On mixing solutions of calcium caseinate and lactalbumin with or without the addition of fat, the essential characteristics of the foaming properties of the corresponding separated milk can be duplicated (figure 2). On the other hand, the addition of lactoglobulin to a calcium caseinate solution does not alter the foaming properties of the latter to any great extent (table 1).

(d) At temperatures below 30° C., skim milk gives a more stable foam than the acid or rennet wheys separated from it. This is due to the absence of calcium caseinate from the whey.

The conclusion is contrary to the prevailing ideas in the dairy field, but it is further justified by the data of other workers. Sharp, Myers and Guthrie (20) investigated the accumulation of protein in the foam of skim milk and came to the conclusion that "there is no preferential accumulation

of any major protein fraction in the foam." Table 4, compiled from their data, indicates that there is a preferential adsorption of casein in the foam at 10° C. which is in complete agreement with the present conclusion.

The preferential adsorption of the substance having the highest surface activity at the air solution interface is in accord with thermodynamical principles and has been reported by many investigators (2, 6, 7, 8, 16, 23, 24). Other workers have found this principle useful in biochemical studies (13, 14).

Ansbacher, Flanigan and Supplee (1) eluted commercial casein with sodium chloride and claimed that they obtained the foaming substances from casein, since the eluted casein did not foam appreciably. They did not, however, present data on this foaming substance or of the casein before and after elution. In a previous paper (5a) we showed that when casein is eluted with 4 per cent NaCl at pH 4.2, the foaming properties of the calcium caseinate prepared were not reduced. Considering that casein at its isoelectric point is soluble in 0.115 N NaCl solutions to the extent of 3.46 gm. per liter (17) and that casein has its maximum foaming capacity at a concentration of about 0.05 per cent, the complications in the studies of these workers may be realized.

Another attempt to assign the foaming properties of separated milk to a minor fraction of its proteins has been reported recently by Sharp and Krukovsky (19). These workers reported two paradoxical ideas; while they did not think that less efficient separation and consequently higher fat content in the skim milk separated at low temperature was the cause of its poor foaming properties, they reported that when this separated milk was extracted with petroleum ether its foaming properties then approached those of the high temperature separated milk. This result would seem to indicate the presence of an anti-foamer (lipoid) prior to extraction but not the absence of an agglutinin. Our results show the "agglutinin" is not important in the foaming of separated milk.

#### SUMMARY

Synthetic solutions of milk proteins and fat have been prepared which duplicate the essential foaming characteristics of whey and skim milk. Lactalbumin and milk fat were found to be the constituents that influence the foaming of whey. The constituents in milk or skim milk that determine its foaming properties are shown to be calcium caseinate, milk fat and lactalbumin. It is considered that calcium caseinate is preferentially adsorbed at the air/liquid interface at temperatures below the melting point of milk fat, lactalbumin being adsorbed at higher temperatures. The presence of milk fat globules is shown to be responsible for the minimum foaming of separated milk at certain temperatures. The addition of milk fat emulsion to blood serum brings about a minimum foaming at room temperatures.

vious work which is not in agreement with the present conclusion is analyzed in the light of the present studies.

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# A STUDY OF METHODS OF OBTAINING MILK SAMPLES FOR ESTIMATING MILK FAT BY THE MOJONNIER METHOD

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Wide differences in the amounts of milk fat were obtained from some samples of the same milk by the Babcock and by the Mojonnier methods. An examination of the data revealed that the method of weighing the samples of milk and of transferring them into the extraction flask by the Mojonnier method was responsible for these abnormal variations.

Though not recognized as official, the Mojonnier method is used extensively in dairy laboratories in this country instead of the Røese-Gottlieb method (1). Some essential details of the technic for obtaining the sample of milk are lacking in the Røese-Gottlieb method (1) and three different techniques are used in the Mojonnier procedure (3, 4).

The writers were unable to find any data in the literature on this subject, except a statement by Dahlberg, Holm and Troy (2) which is quoted. "It was thought that the measuring of samples would introduce slight errors and that fat might rise in the weighing pipette during the time required for weighing to such an extent that extra rich milk might remain in the pipette after the sample was delivered. Both practices were discontinued. After the samples had been heated and mixed until they appeared homogeneous, the milk required for one analysis was quickly poured out and weighed. The samples were weighed directly into the Mojonnier tubes at one laboratory, and at the other, they were weighed into small 25-cc. flasks and transferred to Röhrig tubes, using all of the chemicals used in the test to rinse out the flasks." Therefore it was deemed advisable to compare the different methods of transferring and of weighing samples of milk into Mojonnier extraction flasks.

## PROCEDURE

The milk samples were heated to 35 to 37.5° C. in a water bath at 41.5 to 43° C., and mixed by pouring three times from one container to another. The pipettes in all three methods were filled so that the bottom of the menisci were level with the mark on the draw tubes. Each pipette delivered slightly less than 10 grams of milk at 35–37.5° C.

In method one, four pipettes were filled, the milk sample being poured once into another container between the filling of each pipette. As each

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pipette was filled, it was placed on a holder and the holder with the filled pipettes was placed on the balance pan. When the total weight was obtained, pipette number 1 was emptied into extraction flask number 1 and allowed to drain for 10 to 15 seconds. This empty pipette was again placed on the holder and the weight of milk delivered into the flask was obtained by difference. This procedure was repeated to obtain the weight of milk delivered by pipettes number 2, 3 and 4 into each of three extraction flasks that were similarly numbered. The weight of the four pipettes full of milk and the weighing stand is about 112.5 grams.

In method two, a charge of milk, using pipette number 1, was obtained from the sample, transferred immediately to a tared extraction flask, allowed to drain for 10 to 15 seconds after the flow had ceased and weighed. This was done in duplicate on each sample. The weight of the flask and charge of milk is about 76.1 grams.

In method three, a charge from the milk sample, with pipette number 1, was transferred volumetrically to an extraction flask and allowed to drain for 10 to 15 seconds after the flow had ceased. This was done in duplicate on each sample. In recording the results it is assumed that the pipette delivered 10 grams of milk.

Composite milk samples preserved with bichloride of mercury were obtained from two nearby milk plants and were sampled and transferred by methods one and two and estimated for milk fat. These plants are designated A and B. Plant A had excellent facilities for storing the composites during the bi-monthly period. They were kept constantly in a refrigerated compartment at 10° C. or lower. At the end of the bi-monthly period the samples were free of mold growth and in excellent physical condition. Plant B did not have such good facilities. The preserved milk composites were heavily contaminated with mold at the end of the bi-monthly period and the fat emulsion showed varying degrees of destabilization when tested at the milk plant. These composites were heated and prepared for testing the same as the unpreserved samples except that the cream adhering to the inside of the sample bottle was brushed loose. These preserved samples had been prepared and estimated once for milk fat by the Babcock method in the milk plant before they were brought to the Experiment Station laboratory. All the samples were selected, insofar as possible, to represent high, medium and low fat content milks in each trial.

The Mojonnier (3, 4) gravimetric procedure, equipment and prescribed reagents were used to estimate the milk fat. This is a mechanized modification of the Röse-Gottlieb method. Blank determinations were made on new supplies of reagents. The ether extractions on replicate samples obtained by the three methods were made at the same time. The temperature of the laboratory and equipment did not vary greatly from 21° C., the importance of temperature having been emphasized by Mojonnier (3) and Troy (6).



## RESULTS

The results in table 1 show the effect of three methods of weighing and transferring 30 unpreserved and 120 preserved milk samples into extraction flasks. When milk samples are weighed in pipettes on the weighing stand (method one), there is a gradual decrease in the amount of milk fat from

TABLE 1

*The effect of different methods of transferring preserved and unpreserved milk samples into extraction flasks on the estimation of milk fat by the Mojonnier method*

Number of samples	Mean per cent milk fat							
	Weighed in						Measured in	
	Pipettes—Method one				Flasks—Method two	Flasks—Method three		
	1	2	3	4				
Unpreserved								
10	4.6126	4.5746	4.5260	4.5156	4.6222	4.6097	4.5849	4.5800
10	3.6572	3.6386	3.5859	3.5891	3.6949	3.6723	3.6449	3.6604
10	3.3385	3.3129	3.2897	3.2900	3.3550	3.3411	3.3225	3.3134
Preserved Plant A First Trial								
10	4.2701	4.2293	4.1541	4.1311	4.2991	4.3061		
10	3.5434	3.5395	3.4976	3.4539	3.5693	3.5680		
10	3.2142	3.1927	3.1491	3.1423	3.2332	3.2363		
Second Trial								
10	4.2718	4.2490	4.1845	4.1625	4.3031	4.2934		
10	3.5710	3.5564	3.5135	3.4994	3.6061	3.6076		
10	3.1420	3.1311	3.0821	3.0804	3.1731	3.1782		
Plant B First Trial								
10	4.5192	4.4844	4.3808	4.3876	4.6055	4.5980		
10	3.8707	3.8611	3.7870	3.7568	3.9230	3.9130		
10	3.4147	3.4008	3.3496	3.3371	3.4533	3.4482		
Second Trial								
10	4.7224	4.7059	4.6517	4.6315	4.7990	4.8054		
10	3.8834	3.8814	3.7996	3.7874	3.9421	3.9413		
10	3.5008	3.5010	3.4392	3.4348	3.5499	3.5538		

pipettes 1 to 4. The results from pipette number 1 (method one) agreed closely, but averaged slightly lower than those where the samples were weighed directly into the extraction flasks (method two). Measuring milk by volume and assuming that the pipette delivered 10 grams of milk, yielded lower results than weighing it into extraction flasks. Pipette number 1

delivered an average of 9.9402, 9.9200 and 9.9314 grams of the high, medium and low fat unpreserved milks, respectively, at 35–37.5° C. When calculations are made for the weight of the unpreserved milk delivered by this pipette, then the results obtained by measuring and by weighing milk into extraction flasks agree closely.

The results obtained by weighing the samples from Plant A into extraction flasks (method two) are slightly higher than those from pipette number 1 (method one). However, the same comparison from Plant B indicates

TABLE 2

*A distribution of results from the pipettes that yielded the highest estimation of milk fat by method one on replicate samples*

Milk source	Pipette number			
	1	2	3	4
Unpreserved .....	9 9 8	1 1 .....	..... ..... .....	..... ..... 2
Preserved				
Plant A				
First trial .....	8 4 9	2 5 1	..... 1 .....	..... ..... .....
Second trial .....	9 9 10	1 1 .....	..... ..... .....	..... ..... .....
Plant B				
First trial .....	9 6 8	1 4 2	..... ..... .....	..... ..... .....
Second trial .....	8 6 6	2 4 2	..... ..... 1	..... ..... 1
Total .....	118	27	2	3
Per cent of total .....	78.67	18.00	1.33	2.0

that significantly higher results were obtained by method two. This can be attributed to the destabilized condition of the fat emulsion in the preserved milk composites from Plant B resulting in more rapid rising of the destabilized fat in the pipettes.

The distribution of results in table 2 indicates that of the 150 estimations for milk fat that were made in replicate samples with method one, pipette number 1 yielded the highest results on 118 samples, pipette number 2 on 27 samples, pipette number 3 on 2 samples and pipette number 4 on 3 samples. The percentage distribution is 78.67, 18.00, 1.33 and 2 per cent for pipettes number 1, 2, 3 and 4, respectively. Pipette number 2 yielded the highest results on 10 unpreserved samples from Plant A and on 15 unpreserved

served samples from Plant B. It is evident that method one is not an accurate procedure to use in weighing and transferring milk samples to extraction flasks.

#### DISCUSSION

This study has shown that method one is not an accurate procedure for weighing and transferring samples of milk to the extraction flasks. This method is not reliable because the clusters of fat rise according to Stoke's Law and are adsorbed to the inner surface of the pipettes. The amount of cream thus adsorbed depends on the length of time that the pipettes are on the weighing stand. The amount is least in pipette number 1 and greatest in number 4. The inside diameter of the pipettes is slightly less than two centimeters. The time required to weigh and transfer four pipettes of milk (method one) to the four extraction flasks was 10.3 minutes for one technician and 12.6 minutes for the other. This would be sufficient time for some of the clusters of cream to rise in the pipettes according to the calculations by Sommer (7). In spite of the fact that four charges can be obtained with five weighings, method one is not reliable for whole milk when a high degree of accuracy is desired. The error is not great for pipettes 1 and 2, but is significant for pipettes 3 and 4. Method one was developed by Mojonnier Brothers (3, 4) especially for evaporated milk, sweetened condensed milk, ice cream, and other milk products of a homogeneous nature, but technicians might interpret the instructions to apply this method to the estimation of milk fat in whole milk. The senior author has observed the use of method one on milk in several laboratories. This method saves time because four samples can be obtained with only five weighings, while eight weighings must be made when the samples are transferred directly into the extraction flasks. The errors involved in method one would not have been considered serious twenty years ago, but now are significant with the trend toward greater accuracy and efficiency in the dairy industry. Mojonnier Brothers (5) recently emphasized that the weighing stands with pipettes are intended to be used only for products in which the fat does not readily separate.

Method two is the most reliable. It is possible to obtain a charge of milk from a well-mixed sample and deliver it immediately to the extraction flask. This is especially important in sampling milks where the fat emulsion is partially destabilized, because the destabilized fat rises rapidly in the sample jar as well as in the pipette. Therefore, it is highly important to obtain the sample quickly and deliver it immediately into the extraction flask. Mojonnier (3, 4) recommends this method for products that are not homogeneous or when the milk fat separates rapidly. When method two was used, duplicate determinations for milk fat agreed closely.

Method three may be used in commercial laboratories, provided the pipette is calibrated to deliver a definite charge of milk under standard conditions. The volumetric procedure is recommended by Mojonnier Brothers

(3, 4) but they are careful to specify that their pipettes are calibrated to deliver 10 grams of milk at 15.5° C., allowing 15 seconds for the pipette to drain after the milk has ceased to flow and blowing out the last drop. A sampling temperature of 35–37.5° C., was used in this study, because it was assumed that a more representative sample would be obtained and that the pipettes would drain more completely when the milk fat is liquid. This higher sampling temperature is at least one reason why the pipettes did not deliver the specified 10 grams of milk.

#### CONCLUSIONS

1. Weighing milk in four pipettes on the weighing stand and transferring it to extraction flasks is not an accurate procedure. (Method one.)
2. Weighing milk directly into a tared extraction flask is the most reliable of the three methods. (Method two.)
3. Measuring milk by volume into the extraction flask with a pipette will give sufficiently accurate results for routine work provided the pipette is calibrated for the amount of milk that it will deliver under standardized conditions. (Method three.)
4. For sampling and delivering the milk sample into the extraction flask with a pipette in methods two and three, the thoroughly mixed milk should be at a temperature slightly above the melting point of the fat; for example 35–37° C.

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# CHLORINE RESISTANCE OF *PSEUDOMONAS PUTREFACIENS*\*

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In the attempts to control outbreaks of the putrid defect in butter, chlorine solutions frequently are used to treat butter wash water and sometimes to treat plant equipment, especially churns. The difficulty in controlling the outbreaks with such procedures has suggested to certain plant operators that the causative organisms are relatively resistant to chlorine, although there remains the possibility that the suspected source of the organisms—wash water or plant equipment—was not the actual source so that the organisms were not exposed to the chlorine.

Since *Pseudomonas putrefaciens* is a common cause of the putrid defect in butter (1, 3), its chlorine resistance was investigated. Some of the cultures used were stock cultures, while others were freshly isolated for the study. The problem of chlorine resistance of *Ps. putrefaciens* is greatly complicated by the difficulty in growing the organism (2). In the studies an attempt was made to find the conditions under which it grows best.

The usual procedure was to suspend a 2-day agar culture of *Ps. putrefaciens* in sterile or pasteurized water, allow the suspension to stand for a short time at room temperature and then treat portions with the required amounts of a hypochlorite solution containing 100 ppm. chlorine. After the desired exposures, action of the chlorine was stopped by adding a solution of sodium thiosulfate. Occasionally, two concentrations of chlorine were used on the same original suspension. With each type of water some of the trials included titration of the chlorine at the beginning and end of the exposures; commonly, the chlorine concentration did not decrease significantly during an exposure and the values given represent concentrations at the ends of the exposures. The untreated and treated suspensions were cultured on special agar (2) and in some instances were used to wash butter granules obtained by churning thoroughly pasteurized sweet cream in sterile glass jars; in a few trials they also were cultured in milk.

## RESULTS

*Trials with sterile distilled water.* Addition of 1 ppm. chlorine to a suspension of *Ps. putrefaciens* in sterile distilled water usually resulted in death of the organism in 5 seconds, but occasionally survival was noted for as long as 3 minutes; generally, suspensions requiring longer treatment contained more organisms originally than those requiring shorter treatment although other factors may have been involved. In 10 minutes the organism regularly was killed. With 5 ppm. chlorine the organism never survived a

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TABLE 1  
Action of chlorine on *Ps. putrefaciens* suspended in well water

Trial	Strain of organism	Material examined	<i>Ps. putrefaciens</i> per ml. <sup>3</sup>	Butter washed with material examined†
1	1	Inoc. well water	40,000	Putrid in 2 days
		Inoc. well water + 5 ppm. Cl for 2 min.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 5 min.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 10 min.	< 1	No change in 4 days
2	1	Inoc. well water	30,000	Putrid in 2 days
		Inoc. well water + 5 ppm. Cl for 10 sec.	< 1	Slightly off in 4 days†
		Inoc. well water + 5 ppm. Cl for 30 sec.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 2 min.	< 1	No change in 4 days
3	1	Inoc. well water	54,000	Putrid in 2 days
		Inoc. well water + 5 ppm. Cl for 5 sec.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 10 sec.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 30 sec.	< 1	No change in 4 days
4	2	Inoc. well water	12,000	Putrid in 2 days
		Inoc. well water + 5 ppm. Cl for 5 sec.	< 1	No change in 4 days
		Inoc. well water + 1 ppm. Cl for 30 sec.	840	Putrid in 2 days
		Inoc. well water + 1 ppm. Cl for 2 min.	< 1	No change in 4 days
5	3	Inoc. well water	16,000	No change in 4 days
		Inoc. well water + 1 ppm. Cl for 1 min.	Plates overgrown	No change in 4 days
		Inoc. well water + 1 ppm. Cl for 5 min.	Plates overgrown	No change in 4 days
		Inoc. well water + 1 ppm. Cl for 10 min.	130	No change in 4 days
		Inoc. well water + 1 ppm. Cl for 20 min.	< 1	No change in 4 days

\* As determined by smears on special agar.

† Unsalted butter held at 21° C.

‡ Tests indicated that *Ps. putrefaciens* was not present.

5-second exposure, which was the shortest one used. During trials with this type of water it was noted that without chlorination *Ps. putrefaciens* remained viable for only a few hours. While survival for 1 hour, or even much less, was sufficient for conducting the tests on a suspension, it appeared that killing the organism with chlorine in this type of water would be easier than in the type of water generally used in dairy plants. Accordingly, additional tests were conducted.

*Trials with pasteurized well water.* Water was obtained from the college water system before filtration; after pasteurization at approximately 90° C. for 30 minutes, it was cooled to room temperature and filtered to remove the precipitated material. Table 1 gives representative results obtained with this type of water.

In trial 1 with strain 1 the original suspension of organisms had a count of 40,000 per ml. and 5 ppm. chlorine killed them with exposures of 2, 5 or 10 minutes. The unchlorinated water produced a putrid condition in unsalted butter in 2 days at 21° C. while the chlorinated samples failed to produce the defect. In trials 2 and 3, also with strain 1, satisfactory destruction of the organisms was obtained with 5 ppm. chlorine, even with very short exposures. With an exposure of only 5 seconds (trial 3) no growth was obtained on plates and no spoilage (due to *Ps. putrefaciens*) resulted when the treated water was used to wash butter. The effect of a smaller dosage of chlorine is shown in trial 4 conducted on a suspension of strain 2 containing 12,000 organisms per ml. Although 5 ppm. chlorine for 5 seconds resulted in satisfactory destruction, 1 ppm. for 30 seconds failed to kill all the organisms and unsalted butter washed with the water became putrid in 2 days at 21° C.; however, exposures of 2 or 10 minutes resulted in satisfactory destruction. In trial 5, conducted on a suspension of strain 3 containing 16,000 organisms per ml., 1 ppm. chlorine failed to give as satisfactory destruction as in trial 4 and there was some survival after 10 minutes exposure although all the organisms were killed in 20 minutes; these suspensions were not tested in experimental churnings. Presumably strain 3 was comparatively resistant to chlorine.

The data indicate that *Ps. putrefaciens* is rather susceptible to destruction by chlorine, being rapidly killed, even when present in comparatively large numbers, with a concentration of 5 ppm.. With less chlorine more time is required, but again the organism displays no unusual powers of resistance. Under certain conditions, such as presence of organic matter in the water, excessive numbers of organisms or some comparable factor, less satisfactory destruction would be expected. The type of chlorine compound and the pH at which it acts need definite consideration.

*Trials involving unsatisfactory destruction of Ps. putrefaciens.* In certain trials chlorine failed to give satisfactory destruction of *Ps. putrefaciens*, even when allowed to act for considerable periods; the following examples illustrate this.

Example 1. A suspension of 480,000 organisms per ml. in lake water (pasteurized and filtered) was treated with sufficient chlorine to give a residual of 0.9 ppm. after 2 minutes. Under these conditions the chlorine failed to kill enough organisms in 5 seconds, 10 seconds, 1 minute or 2 minutes to show any destruction on agar plates smeared with 1 ml. or 0.1 ml. of the treated suspensions. In addition, all the chlorinated samples produced spoilage in experimental butter in the same period as the unchlorinated suspension.

Example 2. Well water (pasteurized and filtered) with a count of 620,000 organisms per ml. was treated with 1 ppm. chlorine for periods up to 2 minutes. All the exposures used failed to give appreciable decreases in the number of organisms. In another trial with well water containing 60,000 organisms per ml., 2 ppm. chlorine failed to give satisfactory destruction even after 10 minutes.

Presumably, various factors are involved in the low destruction of *Ps. putrefaciens* in certain trials. In example 1 the number of organisms was excessive for the amount of chlorine and the periods of action; in addition, the water evidently contained considerable organic matter since chlorine was rapidly dissipated in it without any addition of organisms. In the first trial of example 2 an excessive number of organisms in relation to the amount of chlorine and periods of action was again involved. However, in the second trial this apparently was not the case although the strain employed was a very recent isolation which may have grown poorly on the agar so that the count obtained was much lower than the actual number of cells present; apparently, the strain was not especially resistant because in later trials it was easily destroyed by chlorine.

*Growth of Ps. putrefaciens in the test media employed.* When the untreated and treated suspensions of *Ps. putrefaciens* were cultured on the surface of the special agar, growth usually was satisfactory but in a few instances suspensions failed to give the growth expected on the basis of the amount of inoculum used. This was particularly true with certain strains of *Ps. putrefaciens* and especially with some very recent isolations.

Comparisons of growth of *Ps. putrefaciens* on the special agar and in litmus milk indicated that the agar was the better medium. Often suspensions giving good growth on the agar failed to produce any change in litmus milk, even with comparatively large inoculations. There was an excellent agreement between growth of the organism on the agar and production of the putrid defect in unsalted butter at 21° C. Even when a suspension gave comparatively little growth on plates it eventually caused the defect in butter. Commonly, results were obtained more quickly on the agar, where colonies usually were evident in 24 to 30 hours, than in butter; in most cases 2 days or longer were required for production of the putrid defect in butter, even when the wash water contained many organisms.



On the basis of the results it appears that culturing on the special agar is a satisfactory method of measuring destruction of *Ps. putrefaciens* although even on this medium some cells apparently fail to grow. The agreement between growth on the agar and production of the putrid defect in butter is significant from the practical standpoint since satisfactory destruction of the organism has been attained when it does not produce a defect in butter.

#### DISCUSSION

Failure of *Ps. putrefaciens* to show unusual chlorine resistance would be expected because of the absence of spores and the general lack of resistance of the species of the genus *Pseudomonas* to various agents causing destruction of bacteria. When unsatisfactory destruction occurred, the cause generally was apparent and involved factors other than resistance of the organism to chlorine.

The excellent agreement between growth of *Ps. putrefaciens* on the special agar and in butter is rather surprising since it often is assumed that butter is the best medium for the organism. The comparatively poor growth in litmus milk has been noted in other investigations (4).

#### SUMMARY

When suspended in sterile distilled or pasteurized and filtered well water, *Ps. putrefaciens* was easily destroyed by chlorine, provided excessive numbers of cells were not present; destruction was especially active in the distilled water. When the numbers of organisms in relation to the amount of chlorine or its period of action were excessive, destruction was unsatisfactory. Among the strains tested there was some evidence of variation in chlorine resistance.

There was excellent agreement between growth of *Ps. putrefaciens* on a special agar and production of the putrid defect in unsalted butter at 21° C. Various suspensions yielding good growth on the agar failed to produce changes in litmus milk.

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## OBSERVATIONS ON FISHINESS IN BUTTER\*

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At one time fishiness in butter was a common and conspicuous defect. Regardless of the original quality, butter rather frequently developed a pronounced fishy flavor, especially during storage, and the financial losses involved were large. Because of its importance, the defect has been extensively investigated and the more significant factors favoring its development are now generally recognized (2, 3, 4, 5).

While at present fishiness in butter is being controlled to a large extent, the defect still is occasionally encountered. Often this fishiness is rather mild and the pronounced fishy flavors that at one time caused such serious financial losses are unusual. The mild fishiness is not generally recognized as such by the consumer, and the criticism may simply be that the butter is off in flavor.

For the most part the fishiness now encountered in butter is due to failure to apply the control procedures that have been thoroughly established. Illustrations of such failures are reported herein; all of them involve salted butter, fishiness being rare in unsalted butter.

### RESULTS

*Fishiness with low pH values.* In all probability reduction of the acidities at which cream is churned has been the most important factor in limiting the number of fishy churnings and the intensity of the fishiness in those churnings. However, it appears that insufficient acid reduction still is of significance as a cause of the defect.

Table 1 gives the pH values of fishy and non-fishy butter from various plants; the churnings in each series were made within a very short period so that the general conditions of manufacture were much the same, but since each plant obtained cream from a relatively large area there undoubtedly were definite differences in the cream used. In each series the churning or churnings of butter having a fishy flavor had the lowest pH value or values. In some series the difference in pH between a fishy and a non-fishy churning was small.

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TABLE 1

*The pH values of fishy and non-fishy lots of commercial butter from various plants*  
 The 2 to 6 samples from a plant were made during a short period.

Plant no.	Sample no.	Fishy flavor	pH	Plant no.	Sample no.	Fishy flavor	pH
1	1	—	6.75	5	1	—	6.76
	2	+	6.05		2	+	6.37
	3	—	6.56		3	—	6.93
	4	—	6.80		4	+	6.22
2	1	+	6.45		5	+	6.46
	2	+	6.58		6	—	7.24
	3	—	7.04	6	1	—	6.97
	4	—	6.82		2	+	6.51
3	1	—	7.32		3	—	6.91
	2	+	6.58		4	+	6.47
	3	—	7.64	7	1	—	7.02
	4	—	7.08		2	+	6.55
4	1	—	6.96		3	—	6.67
	2	—	6.78	8	1	+	6.68
	3	+	6.52		2	—	7.02
	4	+	6.64				

Distribution of the pH values of a number of fishy and non-fishy churnings of butter from each of four plants is shown in figure 1. The churnings from each plant were not necessarily consecutive but were made within a comparatively short period. In general, the fishy churnings from a plant are grouped among the churnings having relatively low pH values. Also,

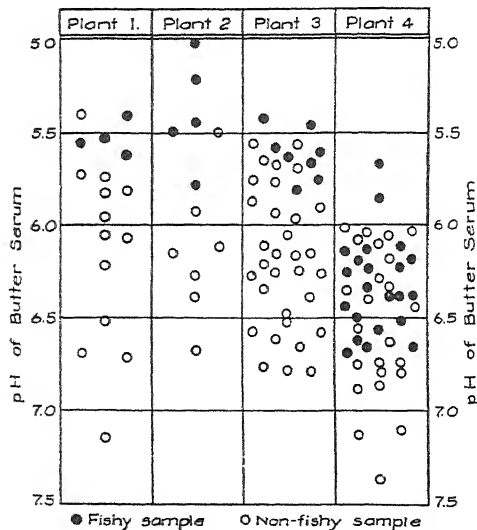


FIG. 1. Distribution of pH values of fishy and non-fishy samples of butter from four plants.

the butter from plant 4 commonly developed fishiness at higher pH values than the butter from the other three plants, suggesting that a factor other than pH also was operative.

The pH values of miscellaneous samples of fishy butter from widely scattered sources are given in table 2. In general, the values are lower than those desired in butter by various manufacturers who attempt to control the pH of their butter within rather narrow limits because of the effect of low pH on chemical deterioration in butter. Some of the values are so low that fishiness definitely would be expected to develop in the butter.

*Fishiness with relatively high pH values.* Occasionally lots of fishy butter have pH values which ordinarily would be considered high enough to prevent development of the defect since they definitely are above the values commonly associated with fishy butter. Because of the relationship of copper to chemical deterioration in butter, a number of such samples were

TABLE 2

*The pH values of miscellaneous samples of fishy butter from widely scattered sources*

Sample no.	pH	Sample no.	pH
1	5.61	11	5.35
2	5.98	12	5.95
3	5.75	13	6.15
4	5.45	14	6.14
5	6.50	15	5.98
6	6.74	16	5.88
7	6.63	17	6.44
8	6.51	18	5.68
9	6.60	19	5.35
10	5.10	20	5.80

examined for copper content, using wet ashing (6) and the carbamate procedure (1, 6). High values commonly were obtained.

The general situation is illustrated by the following case history: In a plant receiving cream from a wide area, special attention was being given to the control of pH in the finished butter, and for the most part variations in pH values of the different churnings were small. At one of the routine examinations of samples from successive churnings in the plant, the flavor scores were normal except in the case of one sample which was decidedly fishy; the samples had been held for about 3 weeks at about 7° C. so that conditions were favorable for development of the defect. It was assumed that the pH of the sample was low, but determinations indicated a value of 7.1. Copper determinations were then made and showed a content of 7.3 ppm. Other churnings made on the same day as the fishy butter contained from 0.4 to 0.75 ppm. of copper.

The plant records for the day on which the defective butter was made showed that the vat of cream used for the butter had a conspicuously lower

acidity than any of the other vats. It included a large proportion of cream from cheese factories, both that from whey and that from milk; this type of cream was reaching the plant at lower acidities than cream coming from farms, either directly or through cream stations. In various cheese factories exposed copper on equipment undoubtedly accounted for a high copper content in the whey and in the cream obtained from it.

Table 3 gives copper contents of additional samples of fishy butter having pH values so high that fishiness ordinarily would not be expected in the butter. The contents vary from 0.85 to 7.0 ppm. While data on the normal copper contents of butter in the areas involved are not available, the values given are definitely higher than those on other churnings from the same plants at approximately the same periods.

TABLE 3  
*Copper contents of samples of fishy butter having relatively high pH values*

Sample no.	pH	Cu content
		<i>ppm.</i>
1	6.70	1.3
2	6.70	1.9
3	7.19	1.3
4	7.25	7.0
5	6.85	2.2
6	7.10	1.9
7	7.13	0.85

In a number of instances fishy butter was encountered which had both a high copper content and a relatively low pH value. Under such conditions the effect of the copper probably was greater than it would have been with a high pH value.

*Control of the pH of butter.* Adequate control of the pH of salted butter, which is so necessary under present conditions of butter manufacture, sometimes presents difficulties. Proper adjustment of the acidity of the cream at the time of churning is a common basis for such control. Under a given set of plant conditions there is a reasonably close correlation between cream acidity at churning and pH of the butter but, because of the influence of various factors on the relationship, results obtained in one plant must be tested in another plant before they can be accepted there. Also, in a plant in which the correlation has been thoroughly investigated an unusual condition may upset the relationship which has prevailed for an extended period.

In a plant which began to receive considerable volumes of cheese factory cream, both whey cream and cream from whole milk, it was noted that the butter was developing a fishy flavor soon after manufacture. Study of the butter showed that the pH values were relatively low although the churning acidities had not been changed. Eventually it was learned that certain

cheese factories were skimming a very rich cream and then diluting it with cold water to cool the cream and thin it sufficiently to make handling easier. Addition of the water changed the buffer capacity of the cream so that the pH of the cream at a given acidity was considerably lower than it would otherwise have been.

*Sources of copper in butter.* Equipment in certain manufacturing plants is an obvious source of copper in butter. Exposed copper in various vats, in piping, etc., contributes significant amounts of copper to cream and to the butter made from it. Presumably an alloy containing copper also can be of importance in this connection. The definite trend toward the use of special metals in the making of equipment for various types of dairy plants is a clear recognition of the objectionable effects of copper and certain other metals on dairy products.

There also are less obvious sources of copper in cream and butter, such as exposed copper in cheese plants supplying cream, unusual equipment on dairy farms, etc. Some of these may be difficult to detect because of their distance from the butter plant but should be considered when it appears that such defects as fishiness in butter are caused by excessive amounts of copper in the product.

When replacement of equipment showing exposed copper is impossible for financial or other reasons, retinning limits the copper contamination. Also, butter made from the first cream through the equipment each day should be used in channels in which it will soon be consumed rather than in channels in which consumption may be delayed. It is probable that there is a definite advantage in keeping the pH of the butter comparatively high.

Detailed studies on the normal copper content of butter are greatly needed so that it will be possible to tell when the content has been definitely increased during the collection of cream and its manufacture into butter. There is some evidence that the normal copper content of butter from various areas differs rather widely.

*Distribution of fishiness between fat and serum of butter.* In a number of instances the distribution of fishy flavor between fat and serum of butter was studied by melting the butter and separating the fat and the serum with a separatory funnel. The melted fat was then filtered through paper at about 45° C., and the serum was centrifuged to remove the fat as completely as possible.

The fishy flavor was very conspicuous in the fat, while there was little or no fishiness in the serum. When experienced butter judges were asked to taste the serum and identify the defect in the butter from which the serum came, they commonly could not do so; however, if the fat was not rather completely removed from the serum the fishiness was evident in the serum.

When the filtered butterfat was steam distilled the distillate was very fishy, but even after extended distillation some fishiness was still evident in

the steamed fat. In general, the steaming had no significant effect on the acidity of the fat.

#### DISCUSSION

The data emphasize the importance of proper pH control in the manufacture of butter if fishiness is to be avoided and are in agreement with the fundamental studies that have been reported along this general line. They also show that fishiness still occurs in butter from various plants. Proper control of the pH of butter apparently is not as simple as it sometimes is supposed to be. This is particularly true in plants receiving cream from a variety of sources, some of which may present unusual situations from the standpoint of the relationship between the titration value on the cream and the pH of the resulting butter.

The presence of relatively large amounts of copper in butter developing fishiness when the pH values are satisfactory again is in agreement with the results of the early investigations. While contamination of cream and butter with copper in butter manufacturing plants can be controlled under normal conditions by replacement of equipment, retinning of equipment, etc., it is probable that with plants receiving cream from certain sources the cream sometimes contains excessive amounts of copper when it reaches the plants. This general situation shows the importance of a proper understanding of the conditions under which cream is handled before it comes to the butter plants.

Evidence from various sources indicates that factors other than pH and copper content influence the development of fishiness in butter. In addition to those commonly recognized, the area in which the cream is produced and the cream treatment appear to be of importance on the basis of general observations.

The cream from certain producing areas apparently yields butter which is more susceptible to the development of a fishy flavor than cream from other areas. This could easily be related to the feeds consumed by the cows because of their effect on the composition of the milk, including the content of such minor constituents as lecithin (5). However, there also are other possibilities.

Extensive treatment during the processing of cream for butter also may influence the development of fishiness. For example, certain types of pasteurization may affect the stability of the fat or fat-like constituents.

#### SUMMARY

Fishy butter from different plants commonly had lower pH values than non-fishy butter from the same plants at about the same periods. In various instances in which fishy butter had a relatively high pH value, it contained comparatively large amounts of copper.



Proper control of the pH of butter requires recognition of any unusual condition which develops. Certain sources of copper are very obvious but others, such as exposed copper in cheese plants supplying cream for butter manufacture, are much less obvious.

When fishy butter was separated into fat and serum, the fishy flavor was conspicuous in the fat but there was little or no fishiness in the serum.

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# FINAL REPORT OF THE SUB-COMMITTEE ON THE DETERMINATION OF THE PERCENTAGE OF FAT IN SWEETENED CONDENSED MILK AND EVAPORATED MILK

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The committee has made an attempt to find a satisfactory method for the determination of fat in sweetened condensed milk and evaporated milk where Mojonnier equipment is not available.

The standard method of procedure in conducting the Babcock test is unsuitable for testing dairy products containing added sugar on account of the carbonization of the sugar which makes the reading of the fat column difficult.

Accuracy, simplicity, low cost and the use of standard Babcock equipment were the principal factors considered.

Comparisons were made of a number of methods which have been used in determining the percentage of fat in dairy products, with the Mojonnier method as the standard. Some of the methods investigated were eliminated because the originators did not recommend them for testing sweetened condensed milk and evaporated milk. Others were eliminated because the results were not satisfactory to the committee.

It was found, in making a further investigation, that both the Pennsylvania and the Minnesota methods gave results which agreed closely with the Mojonnier method. It was decided to recommend both of these methods.

## PREPARATION AND AMOUNT OF SAMPLE

### *I. Sweetened Condensed Milk*

To facilitate weighing the sweetened condensed milk into the test bottle, it can be mixed with an equal weight of water. This is done by balancing two beakers on opposite sides of a set of cream or similar scales; approximately 4 ounces of sweetened condensed milk are added to one of the beakers and an equal weight of water added to the other and the contents of the two beakers are thoroughly mixed together and then placed in a stoppered flask.

For both the Pennsylvania and Minnesota methods, weigh 9 grams of this mixture into either a 9-gram, 20 per cent ice cream test bottle or an 8 per

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cent milk test bottle. The results are multiplied by two if a 9-gram ice cream test bottle is used and by four if an 8 per cent milk test bottle is used.

## II. *Evaporated Milk*

In the Pennsylvania method, weigh a 6-gram sample into either an 8 per cent milk test bottle or a 9-gram ice cream test bottle. Multiply the results by three when an 8 per cent milk test bottle is used and by one and one-half when a 9-gram ice cream test bottle is used.

For the Minnesota method, weigh a 9-gram sample into a 9-gram 20 per cent ice cream test bottle.

### DIRECTIONS FOR USING THE PENNSYLVANIA METHOD

#### I. *Equipment*

Regular Babcock equipment and glassware, including ice cream test bottles, are employed.

#### II. *Reagents*

1. Ammonium hydroxide (28–29 per cent  $\text{NH}_3$  is necessary).
2. Normal butyl alcohol (B.P.  $117^\circ \text{C}$ .).
3. Diluted commercial sulphuric acid (specific gravity approximately 1.72–1.74). The acid is diluted by adding  $3\frac{1}{2}$  parts, by volume, of commercial sulphuric acid, specific gravity 1.82–1.83, to one part of water, in a heat resisting container.

#### III. *Procedure*

The same procedure is followed in testing the sweetened condensed milk and evaporated milk even though the weight of sample varies. The procedure is as follows:

1. Add 2 milliliters of ammonium hydroxide from a burette.
2. Mix for approximately one-half minute.
3. Add 3 milliliters of butyl alcohol from a burette.
4. Mix for approximately one minute.
5. Add 17.5 milliliters of the diluted sulphuric acid.
6. Mix thoroughly until digestion is completed.
7. Centrifuge the bottles for 5 minutes.
8. Add water ( $130\text{--}140^\circ \text{F}$ .) to bring the contents to within one-fourth inch of the base of the neck of the bottle.
9. Centrifuge for two minutes.
10. Add enough water ( $130\text{--}140^\circ \text{F}$ .) to keep the fat within the graduated portion of the neck of the bottle until read.
11. Centrifuge one minute.
12. Place the bottles in a water bath at  $130^\circ \text{F}$ .
13. Allow a few drops of glymol to run down the inside of the neck of the bottle just before reading. Measure the length of the fat column from the

bottom of the lower meniscus to the sharp line of demarcation between the glymol and the fat.

#### DIRECTIONS FOR USING THE MINNESOTA METHOD

##### I. *Equipment*

Regular Babcock equipment and glassware, including ice cream test bottles, are employed.

##### II. *Reagents*

Minnesota Babcock reagent.

##### III. *Procedure*

The same procedure is followed in testing the sweetened condensed milk and evaporated milk, even though the weight of sample varies. The procedure is as follows as recommended by Thurston and Brown:\*

1. Add 15 milliliters of Minnesota reagent.
2. Shake thoroughly.
3. Digest 12 to 15 minutes in a gently boiling water bath, having the bottles in a rack held at least 2 inches above the bottom of the bath.
4. Shake the mixture in the test bottle vigorously at the time when at least half the contents of the bottle have turned dark brown (usually about  $2\frac{1}{2}$  minutes after placing them in the water bath).
5. Shake vigorously again about one minute later. (Note: Some care may be necessary when starting to shake the bottles the second time, as the alcohol in the reagent may boil off through the neck of the bottle, taking with it some of the mixture.)
6. Place the tests in a centrifuge and centrifuge them for one-half minute at the speed used for the Babcock test.
7. Add hot water ( $130\text{--}140^{\circ}\text{ F.}$ ) to float the butterfat well up into the neck of the test bottle.
8. Centrifuge for one-half minute.
9. Place the tests in a water bath at  $133\text{--}137^{\circ}\text{ F.}$  and leave for 5 minutes.
10. Just before reading each test, allow colored reading fluid (such as glymol) to flow gently onto the surface of the fat column.
11. Hold the bottles in a level position and read as one would read a Babcock cream test.

#### REMARKS

Either method can be expected to give reliable results within the accuracy of reading the calibrations on the Babcock test bottle and both are well adapted to the determination of fat in sweetened condensed milk and evaporated milk.

\* Ice Cream Field, February 1937.



# THE VITAMIN A REQUIREMENTS OF DAIRY COWS FOR PRODUCTION OF BUTTERFAT OF HIGH VITAMIN A VALUE.

## II. VITAMIN A *PER SE*\*

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In previous experiments (8) it was found that the vitamin A requirement of dairy cows for the production of milk fat with maximum vitamin A value was approximately 550,000 units per day when the source of vitamin A in the ration was artificially dried alfalfa hay (carotene). Recent experiments have been completed to determine this daily requirement when the source of vitamin A in the ration was vitamin A *per se* (fish liver oil).

### EXPERIMENTAL

The general plan of procedure in all of these experiments has been to decrease the vitamin A potency of the milk secreted by the cows to a low level by feeding vitamin A deficient rations and then determine the number of vitamin A units required daily by the cows to restore the vitamin potency of the milk fat to a high level.

The vitamin A deficient ration was composed of beet pulp, and a grain mixture consisting of white corn, oats, linseed oil meal, bone meal and salt. The source of vitamin A in the repletion rations was vitamin A *per se* (fish liver oil).

Throughout this report vitamin A activity is quantitatively expressed as Sherman-Munsell units.

#### *Experiment I*

Two Guernsey cows in the early stage of lactation were used in this trial. The cows were fed the vitamin A deficient ration until the vitamin A potency of their milk fat dropped to a low level (12 units per gram). Beginning at this point each cow was fed vitamin A doses of 25,000, 50,000, 75,000, 100,000, and 200,000 units daily in successive 21 day feeding periods.

Representative samples of milk were collected from each cow during the last two days of each period from which butter samples were prepared for biological assay. Each sample of butter was assayed separately for vitamin A potency.

The results of experiment I are shown in table 1 and figure 1.

#### *Experiment II*

Two Guernsey cows were used in the feeding trials of this experiment. After their stores of vitamin A had been depleted to a low level, one cow

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TABLE 1

Showing the vitamin A requirement of dairy cows when vitamin A *per se* was the source of vitamin A in the ration

Trial 1

Period No.	Daily vitamin A unit* intake	Vitamin A in butter units*/gm.	
		Cow 521	Cow 535
1	Depletion	12	12
2	25,000	10	12
3	50,000	11	12
4	75,000	15	17
5	100,000	30	30
6	200,000	36	34

\* Sherman-Munsell units.

was fed daily vitamin A *per se* in units ranging from 50,000 up to 300,000 and back to 50,000 in successive 21 day feeding periods. The other cow, at the end of depletion period, was started on 300,000 vitamin units daily with descending and then ascending quantities fed in successive feeding periods. The feeding schedule of the two cows is shown in table 2.

#### DISCUSSION

As has been stated previously (8) the criterion for the measurement of the vitamin A requirements of dairy cows for the secretion of milk fat with maximum vitamin A value is based upon the supposition that cows are not able to secrete butterfat of maximum vitamin A value until the optimum requirements for maintenance and production have been satisfied. Therefore, the minimum vitamin A intake which will produce the maximum effect upon the milk fat secreted should be the minimum vitamin A requirement of the cow for the production of milk fat of maximum vitamin A value.

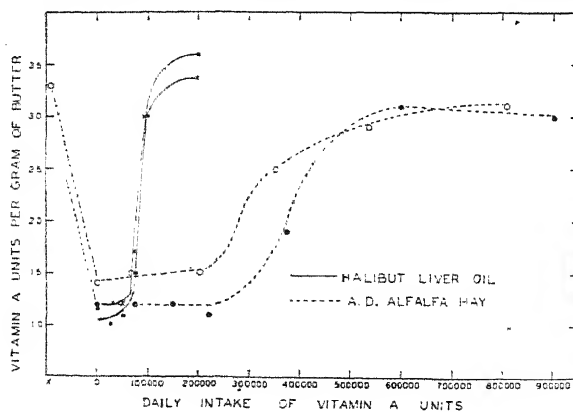


FIG. 1. Showing the relative efficiency of vitamin A *per se* and carotene (artificially dried alfalfa hay) for the production of butter of maximum vitamin A value.



TABLE 2

Showing the vitamin A requirements of dairy cows when vitamin A *per se* was the source of vitamin A in the ration

Period No.	Trial 2—Cow 534		Trial 3—Cow 535	
	Daily vitamin A unit* intake	Vitamin A in butter units*/gm.	Daily vitamin A unit* intake	Vitamin A in butter units*/gm.
1	Depletion	12	Depletion	15
2	50,000	15	300,000	33
3	75,000	18	200,000	33
4	100,000	24	150,000	30
5	150,000	30	100,000	28
6	200,000	32	75,000	21
7	300,000	35	50,000	14
8	200,000	34	0	14
9	75,000	27	100,000	27
10	0	16	200,000	31

\* Sherman-Munsell units.

Previous tests have shown that when dehydrated alfalfa hay was used as a source of vitamin A (carotene) the cows required approximately 550,000 vitamin A units daily to restore the vitamin A potency of the butterfat to its highest level.

In the present tests when the source of vitamin A in the ration was vitamin A *per se* (fish liver oil), these requirements were satisfied with approximately 200,000 units daily. For simplicity in showing the relative efficiency of these two sources of vitamin A, the daily unit intake required of each is shown in figure 1. Data for the carotene (dehydrated alfalfa hay) requirements are taken from previous experiments (8).

In trial I, it became evident that the vitamin A requirement for the production of milk of high vitamin A value was between 150,000 and 200,000 units daily when the source of vitamin A in the ration was vitamin A *per se*. In order to check these results and to determine the requirements as affected by depletion and repletion of body storage trials 2 and 3 were conducted.

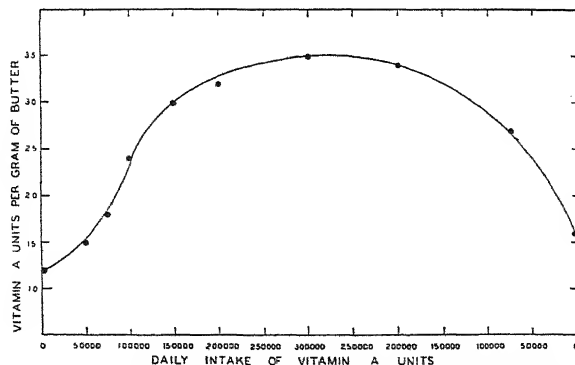


Fig. 2. Showing the vitamin A *per se* requirements for the production of butter with maximum vitamin A value.

As shown in table 2 and figures 2 and 3 the results of the second experiment were almost identical with those of the first experiment. As can be seen in figure 2 when the vitamin A *per se* in the ration was increased from 50,000 to 100,000 units daily there was a significant increase in the vitamin A potency of the milk fat secreted by the cows. The vitamin A value of the milk fat reached its maximum value when the ration contained approximately 200,000 units daily. Increasing the daily vitamin A intake to 300,000 units failed to produce any significant increase in the amount of vitamin A in the milk fat. On the other hand when the amount of vitamin A in the ration was decreased below 200,000 units daily in successive 21-day periods there was a corresponding decrease in the vitamin A potency of the milk fat secreted by the cows. There was, however, a slight lag during the depletion which was probably due to the buffering action of reserve body stores which had accumulated on the high levels.

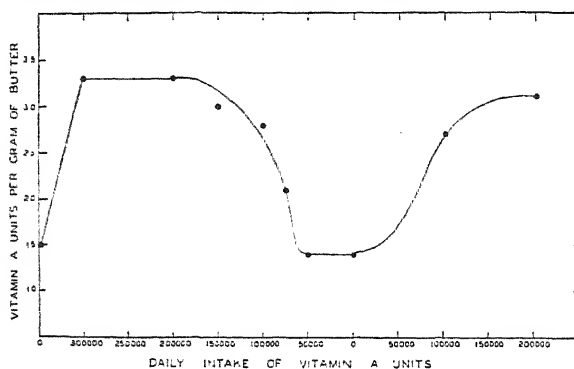


Fig. 3. Showing the vitamin A *per se* requirements for the production of butter with maximum vitamin A value when measured by depletion and repletion tests.

When 300,000 units were fed in the first period following a short depletion period the vitamin A potency of the milk fat was restored to its maximum value immediately. This is shown in table 2 and figure 3. Decreasing the unit intake to 200,000 daily failed to have any significant effect on the vitamin A value of the milk fat. With each increment decrease below 200,000 units daily there was a decrease in the vitamin A potency of the milk fat.

Following the period in which the daily unit intake had been reduced to 50,000 units the cow was again placed on the vitamin A deficient ration for 21 days, during which time the vitamin A potency of the milk fat dropped to a low level (14 units). Following this depletion period 100,000 and 200,000 units was again included in the ration in two successive 21-day periods.

As can be seen in table 2 and figure 3 there was a slight lag in the restoration of the vitamin A potency of the milk fat following this depletion period.

This was probably due to the use of a portion of the vitamin A intake for repletion of body tissues. It should be noted, however, that when 200,000 units were fed daily the vitamin A value of the milk fat again approached its maximum value.

It has been generally recognized that there exists a threshold level for the secretion of vitamin A in milk fat above which more vitamin A or carotene in the feed does not affect the vitamin A potency of butterfat (1, 2, 6, 7). On the other hand, Deuel *et al.* (3) failed to show a definite threshold level in their experiments when they reported that the feeding of shark liver oil produced butterfats of exceptionally high vitamin A potencies as determined by colorimetric and spectrophotometric methods. Rusoff *et al.* (7) reinvestigated this problem and found that feeding 2,144,000 units daily failed to raise the vitamin A potency of the milk above the characteristic threshold level when the vitamin A potency was measured by biological assays. Jensen *et al.* (5), while studying the effect of feeding shark liver oil, found a threshold level in the vitamin A content of blood plasma. The results of our experiments indicate that a definite threshold level for vitamin A does exist. Under the conditions of these experiments, a daily intake of approximately 200,000 units of vitamin A *per se* or 550,000 units of carotene are required to reach this threshold level.

The minimum vitamin A requirement of dairy cows for the secretion of butterfat of maximum vitamin A value has been found to be approximately 200,000 units daily when the source of vitamin A in the diet is vitamin A *per se* and 550,000 units when supplied by carotene in dehydrated alfalfa hay. This would indicate that on the basis of daily unit intake, carotene is only about one-third as effective as vitamin A. Similar differences in effectiveness of these two sources of vitamin A have been reported by Guilbert *et al.* (4) when they studied the minimum carotene and vitamin A requirements of cattle, sheep and swine for the prevention of night blindness.

Although there appears to be a difference in the vitamin A requirements of the dairy cow when vitamin A *per se* or carotene are fed, it is conceivable that the actual physiological requirement is the same. The difference in effectiveness may be due to lower metabolic efficiency in the utilization of carotene or possibly to relative unavailability of the carotene in plant tissue.

#### SUMMARY

1. Feeding experiments have been completed to determine the minimum vitamin A requirements of dairy cows for the production of butter of maximum vitamin A value.

2. When the source of vitamin A in the ration was vitamin A *per se* (fish liver oil), this requirement was satisfied with a daily intake of 200,000 units.

3. In this series of experiments, vitamin A *per se* was found to be approximately three times as effective as carotene in dehydrated alfalfa hay.

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# THE VITAMIN A REQUIREMENTS OF DAIRY COWS FOR PRODUCTION OF BUTTERFAT OF HIGH VITAMIN A VALUE.

## III. AVAILABILITY OF CAROTENE IN DEHYDRATED ALFALFA HAY AS COMPARED WITH CAROTENE IN OIL<sup>1</sup>

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In previous reports (4, 9), it has been shown that the apparent vitamin A requirement of dairy cows for the secretion of milk fat with maximum vitamin A value depends upon the source of the vitamin A in the ration. When the source of vitamin A in the ration was carotene from dehydrated alfalfa hay, it was found that the daily requirement was approximately 550,000 Sherman units (9). With vitamin A *per se* this requirement was satisfied with a daily intake of approximately 200,000 units per day (4). Similar differences in the relative efficiency of utilization of carotene and vitamin A by cattle, sheep and swine have been reported by Guilbert *et al.* (2).

Carotene in some plant tissues has been found to be less effective biologically than would be indicated by analyses. Wide variations in the efficiency of utilization of carotene from different plant sources have been reported by Smith and Otis (8) and by Graves (1).

The purpose of this investigation was to compare the availability and efficiency of utilization of crystalline carotene with carotene in dehydrated alfalfa hay for the production of milk fat of high vitamin A value.

### EXPERIMENTAL

Two groups of two Guernsey cows each were used in the feeding trials to determine the relative availability of the carotene in dehydrated alfalfa hay as compared with crystalline carotene in oil. The cows were similar in respect to stage of lactation, milk production and body weights. The body stores of vitamin A in the cows were depleted in a preliminary feeding period by feeding a vitamin A deficient ration consisting of beet pulp and a grain mixture of 400 lbs. white corn, 200 lbs. oats, 150 lbs. linseed oil meal, bone meal and salt. In successive feeding periods, the carotene of the hay and the carotene in the oil were equilibrated at levels of 130, 200, 300, and 200 milligrams daily during the respective feeding periods of 21 days each.

The dehydrated alfalfa hay was sacked immediately after dehydration and stored. Prior to each feeding period, sufficient number of sacks of hay

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for the next feeding period were set aside, carefully sampled, and analyzed for carotene. In preliminary determinations of carotene it was observed that the Peterson-Hughes method (6) gave consistently higher values than the methods of Hegsted, Porter and Peterson (3) and Moore (5). Since in each of these procedures a different principle is involved for the removal of the non-carotene chromogens, tests were made to determine the effectiveness of each procedure. Samples of dehydrated alfalfa hay were extracted according to the Peterson-Hughes procedure. The final petroleum ether extracts were washed with water and made to volume. Critical studies upon aliquots from these solutions disclosed that extraction of the non-carotene pigments with 94 per cent diacetone alcohol or by adsorption upon a dicalcium phosphate column were equally effective and gave carotene values in close agreement, while extraction with 90 per cent methanol failed to remove

TABLE 1

*A comparison of the relative availability of carotene in dehydrated alfalfa hay and crystalline carotene in oil*

Period	Group No.	Carotene supplement*		Butterfat	
		Dehydrated alfalfa hay	Carotene (in oil)	Sample No.	Vitamin A potency
		<i>mg. per day</i>	<i>mg. per day</i>		<i>Sherman units per gram</i>
1	1	None	None	1	11
	2	None	None	2	13
2	1	130	None	3	19
	2	None	130	4	19
3	1	200	None	5	32
	2	None	200	6	34
4	1	300	None	7	37
	2	None	300	8	36
5	1	None	None	9	22
	2	None	None	10	21
6	1	None	200	11	32
	2	200	None	12	33

\* Ration consisted of beet pulp and a grain ration consisting of 400 lbs. white corn, 200 lbs. oats and 150 lbs. linseed oil meal.

some of the pigments and gave high carotene values. When these latter solutions were purified by further treatment with either diacetone or dicalcium phosphate, the carotene values were lowered to agree with the others. Therefore, in these experiments, the carotene values obtained by the Moore procedure (5) were used in calculating the amounts of hay to be fed daily to the cows.

The crystalline carotene in oil was purchased from the National Research Associates, Inc., as a product known as "Research" Carrot Oil which had been prepared from carrots as a thick suspension of crystalline carotene in oil (approximately 3.2 per cent carotene). Upon receipt of the oil, it was removed from the container, thoroughly mixed and bottled in 250-ml. brown bottles under an atmosphere of nitrogen. Samples were taken from a num-

ber of bottles for carotene analyses. These gave concordant results, indicating that the distribution of carotene was uniform. To prevent deterioration, the carrot oil was kept in cold storage until used.

For the carotene analyses, aliquots were dissolved in petroleum ether and the carotene determined with a Bausch and Lomb Spectrophotometer. By passing the petroleum ether solution through a dicalcium phosphate column to remove the non-carotene pigments, it was found to contain 5 per cent impurities. According to the chromatographic analyses which were made by J. W. White, Jr., the carotene was composed of 25.2 per cent alpha-carotene and 74.8 per cent beta-carotene.

Since alpha-carotene possesses but half the biological activity of beta-carotene, it was necessary to equilibrate the carotene of the carrot oil to a biological equivalence of beta-carotene. On the basis of these corrected carotene values, the requisite amount of carrot oil for each feeding dose was weighed into No. 10 gelatin capsules and fed to the cows.

At the end of each feeding period 24-hour composite samples of milk from each group were collected and the cream separated and churned into butter. The butter samples were stored at  $-18^{\circ}\text{C}$ . and the portions were removed as needed for biological assays. The butterfat was heated to  $55^{\circ}\text{C}$ . and filtered to remove curd and water. The results of the biological assays of the butterfats are given in table 1.

#### DISCUSSION

In previous experiments (4, 9), it was found that the carotene of dehydrated alfalfa hay was only about one-third as efficient as vitamin A *per se* as a source of vitamin A in the rations of dairy cows for the production of milk fat of maximum vitamin A value. This difference might be due either to the relative unavailability of carotene in the plant tissue, or if available, to differences in the metabolic efficiencies. A comparison of the availability of crystalline carotene and the carotene in plant tissue should give an insight into this problem.

To test these possibilities, it was imperative that comparisons be made at levels which would be potentially capable of producing differences in the vitamin A potencies of the butterfats. If comparisons were made at too low levels, it is conceivable that differences might exist which would not be reflected in the vitamin A potencies of the butterfat. Likewise, if comparisons were made at levels above the minimum requirements of the cow for the production of butterfat of maximum vitamin A value differences would not be measurable. Therefore, levels of carotene intake were selected which might be expected to be intermediate between these two points.

The results of these experiments, as shown in table 1, indicate that the dairy cow can utilize the carotene in alfalfa hay as readily as isolated carotene for the production of butterfat of high vitamin A value. It is interesting to note that the vitamin A potencies of the butterfats produced by the

two groups on each of the different levels were very similar. This is true even when the two groups were reversed in period 6.

In experiments to determine the minimum carotene requirements of cattle, swine, and sheep for the prevention of night blindness, Guilbert *et al.* (2) found that the requirements were practically the same when the carotene was furnished by alfalfa or by crystalline carotene dissolved in cottonseed oil. In experiments to determine the requirements of chickens for vitamin A when fed as carotene, Sherwood and Fraps (7) found that there was no apparent difference in the effectiveness of carotene supplied in alfalfa meal or by crystalline carotene in oil.

Considering the evidence from all these experiments, it is apparent that the difference in effectiveness of carotene in alfalfa hay and vitamin A *per se* is not due to the unavailability of the carotene in the plant tissue but rather is due to differences in the metabolic efficiencies.

#### SUMMARY

1. Experiments have been conducted to determine the relative availability of carotene in dehydrated alfalfa hay as compared with isolated carotene (in oil) as a source of vitamin A in the rations of dairy cows.

2. Two groups of cows were used in these experiments. Each group of cows was fed equal amounts of carotene at levels of 130, 200, and 300 milligrams daily.

3. The results of these experiments indicate that dairy cows can utilize the carotene from the alfalfa hay as readily as isolated carotene.

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# TEMPERATURE ERRORS IN WEIGHING AND THEIR CONTROL IN THE MOJONNIER FAT TEST

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The Mojonnier test for fat is one of the most accurate methods available for the analysis of dairy products. Nevertheless, analyses of duplicate samples made in different laboratories sometimes differ widely. It is the purpose of this paper to present data concerning one source of error in this test, temperature variations; and to describe an improved method of temperature control for Mojonnier machines.

## EXPERIMENTAL

### *Experiment 1*

It is a common observation that the weight of Mojonnier fat dishes can be reduced by warming. This is easily explained. When the dish is warmed, the air in it expands, and a part of it is expelled from the dish as effectively as if a cover had been placed over it and a part of the air had been removed with a vacuum pump. As a result, the dish will appear lighter in weight. In order to avoid such errors, the temperature of the air inside the dish must be the same as that of the air surrounding the dish.

Quantitative data on the relation between temperature and weight were obtained by weighing dishes first at one temperature and then at another. This was accomplished by placing two Mojonnier machines side by side and adjusting the water in one reservoir so that it was about 3° C. above the temperature of the water in the other machine. Four clean fat dishes were placed in the cooling oven of the warmer machine and, after 10 minutes, each dish was weighed. Immediately after weighing, the dishes were placed in the cooler machine. When all of the dishes had been weighed from the warmer machine, they were re-weighed from the cooler machine. This procedure of weighing the dishes first from one machine and then the other was repeated several times. The data are shown in figure 1. The upper line indicates the successive temperatures at which the dishes were equilibrated. The lower line shows the average weight of the four dishes after equilibrating at the temperature shown above. It is evident that a variation of only one degree in temperature may cause a change of approximately  $\frac{1}{4}$  mg. in weight. This is equivalent to an error of .07 per cent of fat when testing heavy cream.

### *Experiment 2*

It seemed desirable to find a method of reducing errors caused by improper temperature of the cooling oven. Two procedures were tried. First,

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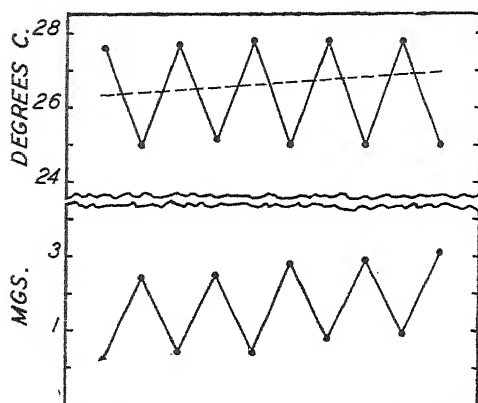


FIG. 1. Variation in the weight of Mojonnier fat dishes (below) due to variation in temperature of the dishes (above). (Actual average weight, 36.0600 grams plus the value shown.)

experiment No. 1 was repeated except that each dish was waved slowly through the air for fifteen seconds immediately before it was weighed. Second, the dishes were removed from the cooling oven and, before weighing, they were placed for two minutes upon a large sheet of  $\frac{1}{16}$ -inch thick aluminum. This sheet was supported only at the corners so that air could circulate around it freely.

The data are presented in figures 2 and 3. It is evident that both procedures reduced the error due to improper machine temperature, but even the second procedure was not entirely adequate.

### Experiment 3

An examination of figure 1 shows that there was a constant difference in weight of about 2 mg., depending upon whether the dishes were weighed

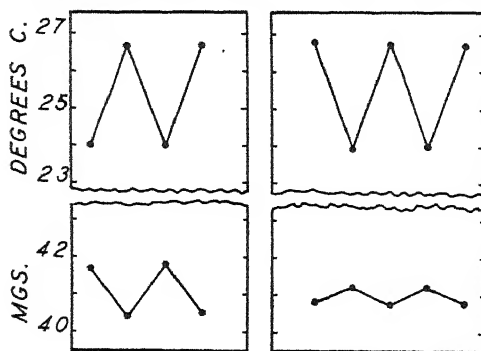


FIG. 2.

FIG. 3.

Variation in the weight of Mojonnier fat dishes (below) after tempering in oven at the temperature indicated above. Figure 2, dishes waved in air fifteen seconds before weighing. Figure 3, dishes left on metal plate for two minutes before weighing.

from the cold or the warm machine. It is evident, also, that all the dishes increased in weight in successive weighings at the same temperature. This slow increase in weight was due to a change in the temperature of the laboratory. The actual change in temperature is recorded by the broken line in the upper part of figure 1. It must be remembered that an error in weight may occur if either the temperature of the room, or the temperature of the dishes, is changed.

The following procedure was used to measure the effect of changes in room temperature upon the weight of fat dishes. A set of four dishes was placed in a Mojonnier machine. The water in this machine was held at a constant temperature while the room was warmed slowly by steam radiators. Three electric fans were used to distribute the heat uniformly through all parts of the laboratory. At intervals, the dishes were removed from the machine, weighed at once, and then returned to the cooling oven. The broken line in figure 4 shows the relation between the weight of the dishes

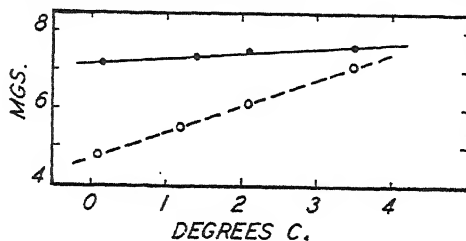


FIG. 4. The relation between the weight of Mojonnier fat dishes and increases in the laboratory temperature. (Machine temperature held constant.) Broken line: weighed direct from cooling oven. Solid line: held on metal plate for two minutes before weighing.

and the increase in the temperature of the room. The water temperature remained constant within  $0.1^{\circ}\text{C}$ .

This experiment was repeated with one change in procedure. Before weighing, each dish was placed on the large aluminum sheet for a period of two minutes. These data are shown by the solid line in figure 4.

#### *Experiment 4*

The Mojonnier machine is designed to bring all dishes to the same temperature before they are weighed. Unfortunately, in most cases, this is not room temperature. In those laboratories where the temperature varies considerably during a 24-hour period, this may lead to appreciable errors. If error is to be avoided, the dishes should be brought to the temperature of the balance, regardless of what that temperature may be, before the dishes are weighed. To accomplish this, some means should be provided for maintaining the cooling oven always at room temperature. It would seem much wiser to circulate the cooling water through an air-cooled radiator than to use a large water reservoir in a Mojonnier machine.

This idea was tested by substituting a small radiator from an automobile heater for the water reservoir of one machine. Air was blown over the radiator by an electric fan. A standard Mojonnier machine was used as a control in comparison with this air-cooled machine.

Two sets of clean fat dishes were used, one set for each machine. The experiment was divided into four periods:

1. All dishes were left on top of the balance case until they showed constant weight. This may be considered the true weight of the dishes, free from temperature errors.

2. Each set of dishes was weighed several times from its own cooling oven to get an average value which was reproducible. Since the cooling ovens had not been adjusted to exactly room temperature, these weights differed from the true weights.

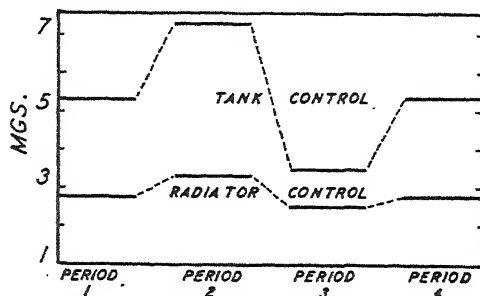


FIG. 5. A comparison of the ability of a tank-controlled machine and a radiator-controlled machine to maintain dishes at constant weight when the room temperature varies. Period 1, dishes at room temperature. Period 2, dishes weighed from cooling oven. Period 3, laboratory cooled  $5^{\circ}\text{C}$ ., dishes again weighed from oven. Period 4, dishes at room temperature ( $5^{\circ}\text{C}$ . lower than during period 1).

3. The laboratory was cooled  $5^{\circ}\text{C}$ . during a period of 90 minutes. Four electric fans were used to maintain uniform temperature distribution throughout the laboratory. When the room had been cooled, the dishes were weighed again from their respective ovens.

4. The fourth and final set of weighings was made after the dishes had rested upon the top of the balance case long enough to come to the temperature of the room. This set of weighings is comparable with the first except that the laboratory had been cooled  $5^{\circ}\text{C}$ .

The data are presented in figure 5. It is evident that the use of an air-cooled radiator in place of a water reservoir on a Mojonnier machine would be quite helpful in any laboratory which is subject to temperature variations.

### Experiment 5

As a further test of the ability of the small radiator to maintain the cooling oven at room temperature, the room was cooled very rapidly, and

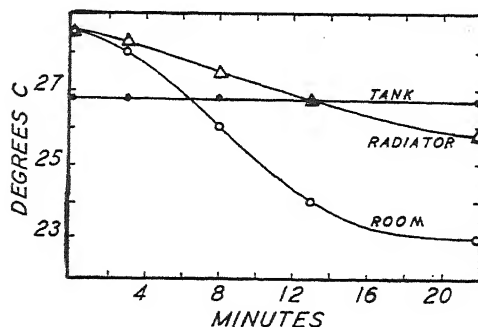


FIG. 6. The comparative rates of temperature adjustment of a tank-controlled machine and a radiator-controlled machine during a period of rapid change in laboratory temperature.

records were kept of the temperature of the room, the temperature of the water circulating through the cooling oven of a standard Mojonnier machine, and the temperature of that circulated through the oven of the air-cooled machine.

The data are shown in figure 6. It is evident that the radiator was too small to keep up with such rapid changes in room temperature though, in this respect, it was far superior to the reservoir on the other machine.

#### Experiment 6

The magnitude of the error due to improper temperature of the dishes is dependent upon the speed with which they are weighed. If the operator were slow enough, the dishes could come to room temperature while he was adjusting weights. In all of the experiments reported thus far, the actual weighing was performed as rapidly as possible, but no time records were kept.

An experiment was carried out to determine the effect of speed of weighing upon the errors in weight. Dishes were removed from an oven which

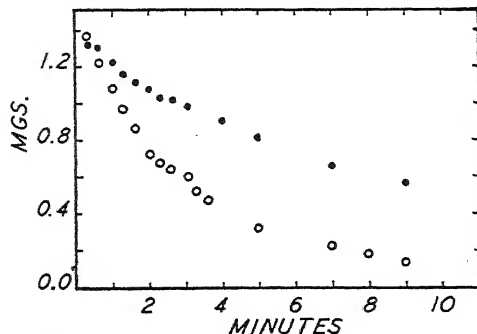


FIG. 7. The rate of cooling of two Mojonnier fat dishes in the balance case as measured by differences between the weight observed at various intervals after removing from the Mojonnier machine and the final equilibrium weight.

was slightly warmer than the room and placed at once upon a balance. Apparent weights were recorded at intervals for thirty minutes, or until the weight remained constant. The final weight was considered the true one, and the errors of earlier weighings were calculated accordingly. Such measurements indicate how slowly the dishes acquire the temperature of the balance case. It was found that different dishes cooled at different rates. Typical data are shown in figure 7. To permit the use of a larger scale in plotting, data are given for only the first ten minutes of the cooling period.

#### SUMMARY

Errors in Mojonnier fat tests may occur whenever the temperature of the room varies during the course of the analysis. Data are presented showing the magnitude of such errors.

It would be preferable to circulate the water for a Mojonnier machine through an air-cooled radiator instead of through a reservoir. Data are given showing the advantages of such a device.

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## ANNOUNCEMENT

Thirty-Ninth Annual Meeting, Ohio State University, Columbus, Ohio.

June 20, 21, 22, 1944

## FIRST CALL FOR TITLES

Titles of original papers to be presented should be in the hands of the Program Committee not later than April 1, 1944. All communications regarding general program plans should be addressed to Professor H. P. Davis, General Program Chairman, Dept. Dairy Husbandry, University of Nebraska, Lincoln, Nebraska. The personnel of the sectional program committees are listed in the preceding list.



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## ACTION OF BUTTER CULTURES IN BUTTER: A REVIEW\*

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*Iowa Agricultural Experiment Station, Ames, Iowa*

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\* Journal Paper No. J-1154 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 127.

## INTRODUCTION

The butter industry early recognized that the type of souring in cream largely determines the flavor of butter freshly prepared from it, and attempts were made to control the souring of raw cream intended for churning by addition of clean-flavored sour milk or cream, buttermilk from a churning of good butter or some similar material. Although pasteurization of cream for butter originally was intended primarily to improve the keeping qualities of the butter, it also permitted better control of the fermentation of the cream through use of selected cultures of bacteria in a medium relatively free of other micro-organisms. Pasteurization of cream and flavor development through use of selected cultures of bacteria increased simultaneously in the butter industry during the final decade of the last century, each tending to complement the other under the general system of butter manufacture then followed. With the recognition of the relationship of high churning acidities in cream to chemical deterioration in butter, use of low acid cream, either sweet or neutralized, and changes in the methods of employing butter cultures were indicated and soon were widely followed.

The present review deals with the general action of butter cultures in butter. It supplements a review (43) dealing with the bacteriology of butter cultures, including the chemical changes brought about in milk through activity of the normal butter culture species.

## METHODS OF USING BUTTER CULTURES IN THE MANUFACTURE OF BUTTER

When butter cultures first came into general use, the common practice was to ripen the cream, either raw or pasteurized, to a comparatively high acidity and then cool and churn. On holding, butter made in this manner sometimes developed serious flavor defects because of the influence of acid on chemical changes in it and, accordingly, methods of employing cultures were developed which involved churning the cream at a relatively low acidity.

The method of using cultures in a plant often is determined largely by the type of butter desired, as well as by the plant equipment and plan of operation. Salted butter for immediate consumption can be churned at a higher acidity, so that it will have more flavor, than butter to be stored for considerable periods during which serious chemical deterioration may occur. With unsalted butter, a high churning acidity ordinarily is employed because in the absence of added salt a high acidity is relatively unimportant as a cause of deterioration; moreover, the flavor desired in unsalted butter intended for table use is best obtained through a high churning acidity.

*Salted Butter*

At present the more common methods of using butter cultures in the making of salted butter are:<sup>1</sup>

<sup>1</sup> A report involving a comparison of several methods of using butter culture is reviewed under the method which gave the best results.

Adding butter culture to pasteurized cream and holding the mixture cold for some hours. An important method of using butter culture is to add 2 to 8 or more per cent to pasteurized and cooled cream, hold the mixture cold for some hours, preferably overnight, and churn. While the added culture raises the acidity of the cream somewhat, there is no significant increase in acid during the holding because the low temperature largely prevents the action of the culture organisms.

Wilster (138) noted the most improvement in the flavor of butter when the cream, with culture added, was held 16 hours below 40° F.,<sup>2</sup> or when the cream was ripened to 0.28 per cent acid (0.42% in the serum) and held overnight below 40° F. With these methods the average scores of the butter were increased nearly 1 point compared to butter made without culture or to butter made with culture added to the cream immediately before churning.

In 28 comparisons, Fabricius and Hammer (33) found addition of 8 per cent culture to sweet or slightly sour cream 16 hours before churning gave butter significantly more often high in score than addition just before churning; this was the case with both the fresh and the stored butter. In 11 trials in which acetylmethylcarbinol plus diacetyl<sup>3</sup> determinations were made on the cream at churning, addition of culture 16 hours before churning commonly resulted in higher values than addition at churning. This was not the case in two of the trials and with these the butter made by adding culture 16 hours before churning scored the lower. In another series of experiments, no culture was added to one lot of cooled sweet cream and to other lots 8 per cent was added at various periods before churning, the periods used in the eight trials including 3, 6, 16, 40, 64, 88 and 112 hours. Butter made with culture consistently scored higher than butter made without culture, both before and after storage. Holding periods greater than 16 hours were of no advantage.  $\text{Amc} + \text{ac}_2$  contents of the cream at churning were sometimes increased and sometimes decreased by holding for the various periods with culture added.

Wiley *et al.* (137) found that addition of 0.5 per cent culture to pasteurized and chilled sweet cream, with holding overnight at a low temperature before churning, improved the flavor of butter. No acid developed in the cream under these conditions, although the resultant butter had a somewhat "brighter" flavor than if made from sweet cream alone.

Fabricius (31) stated that when culture is added to cream at 40° F. or below and the mixture held overnight at a low temperature, considerable  $\text{amc}$  usually is oxidized to  $\text{ac}_2$ . Later (32), he noted that addition of culture to pasteurized and cooled cream several hours before churning brings about

<sup>2</sup> Although in the earlier review (43) temperatures are given on the Centigrade scale, the Fahrenheit scale is employed herein since so many of the results were reported on this basis.

<sup>3</sup> Throughout the remainder of the review  $\text{acteylmethylcarbinol} = \text{amc}$  and  $\text{diacetyl} = \text{ac}_2$ .

little change in the acidity of the cream but usually gives definite increases in the  $\text{amc} + \text{ac}_2$  content.

**Adding butter culture to pasteurized cream and ripening somewhat.** Because of the danger of accelerating chemical deterioration in butter, extensive ripening of cream for salted butter is now rarely used. However, at temperatures favorable for the culture organisms, such as 70° F., slight ripening sometimes is employed, particularly with butter intended for immediate consumption. Ripening of the cream commonly is followed by cooling to a temperature which will largely prevent the action of the culture organisms, after which it is held for some hours before churning.

The Ontario Agricultural College (83) reported that an improved quality of butter for immediate consumption could be made from high acid cream by reducing the acidity, adding a good culture and ripening to a low acidity before churning.

Jackson (52) compared the scores of butter made by adding 10 per cent culture to neutralized and pasteurized cream and ripening for 30 minutes with the scores of butter made from neutralized and pasteurized cream without added culture; he found practically no difference.

White *et al.* (134) stated that ripening cream with culture, even to low acidities, improved the score of the fresh butter. In the experiments of Walts (131) butter made from neutralized and pasteurized cream with culture scored higher in all the trials than butter made without culture. The trials involved ripening the cream 2 hours at 65° F., ripening 12 hours at 65° F., ripening to 0.30 per cent acid at 65° F., ripening 1 hour at 48° to 56° F. and ripening overnight at 50° F.

According to Lucas *et al.* (65) the average score of fresh butter from cream ripened to 0.30 per cent acid was higher than that from cream to which culture was added at the time of churning, that from cream to which culture was added just before working, that from cream ripened to 0.45 per cent acid and that from sweet cream (without culture). Butter from sweet cream lacked flavor and that from cream ripened to 0.45 per cent acid had an undesirable acid flavor; after 30 days in storage, the latter had suggestions of fishy, unclean and tallowy flavors. The authors noted that even under ideal conditions it is unwise to ripen cream with culture, even moderately, if the butter is to be stored for any considerable period.

Price *et al.* (96) compared butter manufactured with and without culture. The culture was added to pasteurized and neutralized cream when it had been cooled to 70° F. After holding at this temperature until 0.28 to 0.30 per cent acid had developed, the cream was cooled to 35° to 40° F., held overnight and churned the following morning. Of 93 churnings, 59 were made with culture added to the cream and 34 were made without culture. The fresh butter made with culture ranged in score from 90.5 to 95

and averaged 93.03; butter made without culture ranged in score from 90.5 to 93.5 and averaged 91.96. After holding 1 month at 35° to 45° F., scores of butter made with culture ranged from 89 to 94 and averaged 92.07; scores of butter made without culture ranged from 89 to 94 and averaged 91.6. After holding 6 months at 0° to 10° F., scores of butter made with culture ranged from 90 to 94 and averaged 92.4; scores of butter made without culture ranged from 89.66 to 93.5 and averaged 91.63. From the frequency distribution of the butter scores, it was noted that 93.23 per cent of the scores of the fresh butter made with culture were 92 and above as compared to 61.77 per cent for fresh butter made without culture. After holding 1 month at 35° to 45° F., 61.02 per cent of the butter made with culture scored 92 and above as compared to 49.98 per cent for butter made without culture. After holding 6 months at 0° to 10° F., 77.97 per cent of the butter made with culture scored 92 and above as compared to 44.12 per cent for butter made without culture. There was very little difference between the keeping qualities of butter made with and without culture. The scores of butter made with culture decreased more during holding 1 month and 6 months, but at the end of each holding period a considerably larger percentage of scores were above 92 than in the case of butter made without culture.

Results of Overman (86, 87) showed that butter having the highest initial score was churned from fresh cream separated from fresh, sweet whole milk. Butter made from pasteurized sweet cream after ripening with culture and partially neutralizing scored higher when fresh than butter made from the same cream after spontaneous souring and neutralizing to the same acidity.

Fabricius and Hammer (33) compared addition of 8 and of 10 per cent culture to cream after cooling with addition of 8 per cent culture to the cream at 70° F., ripening for 1 hour and then cooling. Ripening of the cream gave butter more often high in score than addition of 8 or of 10 per cent culture to the cooled cream, when the butter was fresh, after 6 weeks at 28° F. and after 6 months at 0° F. Differences in score, however, were only significant in the case of the butter held 6 weeks at 28° F. Another series of comparisons involved: (a) Addition of 8 per cent culture to pasteurized and cooled cream with holding at 28° to 36° F.; (b) addition of 8 per cent culture to pasteurized and cooled cream with holding at 41° to 52° F.; and (c) addition of 8 per cent culture to pasteurized cream at 70° F. followed by ripening for 1 hour, cooling and holding at 28° to 36° F. Butter from cream to which 8 per cent culture was added with holding at 41° to 52° F. and from ripened cream was more often higher in score than butter from cream to which 8 per cent culture was added with holding at 28° to 36° F.; however, in no case was the difference significant.

Crossley (21) stated that when growth of the culture organisms is allowed to continue in cream after adding culture, production of  $ac_2$  is in-

creased and full-flavored butter is obtained. The acid developed during ripening, however, has an adverse effect on the keeping qualities of the butter.

Langton (59) noted that cream must be ripened in order to obtain full-flavored butter. *Streptococcus lactis* or *Streptococcus cremoris* plus flavor organisms were considered essential. Various strains of lactic acid streptococci brought about appreciable differences in the flavor of butter.

According to Fabricius (31) when culture is added to cream at 70° F. and the mixture held 2 to 3 hours before cooling, the  $ac_2$  content often decreases and the cream must then develop about 0.40 per cent acid before appreciable amounts of  $ac_2$  are produced.

**Adding butter culture to pasteurized cream at churning.** Butter culture sometimes is added to pasteurized cream at the time of churning. From the standpoint of flavor of the butter, both experimental results and commercial experience indicate that this is less desirable than holding a mixture of cream and culture cold for some hours before churning, but it may be necessary with limited vat space. It also avoids risking an increase in the acidity of the cream-culture mixture when the cooling and holding equipment are inadequate.

Dean (28) noted that the flavor of butter made from sweet cream with culture added at churning was better than that of butter made from sweet cream alone. Sweet cream churned with 27.5 per cent added culture gave better butter than ripened cream when the butter was fresh; it also held its flavor better during storage. Rogers and Gray (104) found that butter made from pasteurized cream with culture added retained its fresh flavor better than ripened cream butter.

Mortensen (80) compared butter made from sweet cream and from sweet cream to which culture was added before churning. Immediately after the butter reached the market, that made from sweet cream scored higher in 8 of 32 trials, that made from sweet cream with culture added scored higher in 16 trials and the scores were the same in 8 trials. After 2 months in cold storage (0° F.), the sweet cream butter had the higher score in 10 of 35 trials, that made from sweet cream with culture added had the higher score in 20 trials and the scores were the same in 5 trials. After 9 months in cold storage, the sweet cream butter had the higher score in 11 of 22 trials, that made from sweet cream with culture added scored higher in 8 trials and the scores were the same in 3 trials. In another experiment, ripened cream butter was compared with butter made from sweet cream to which culture was added without ripening. Immediately after the butter reached the market, the ripened cream product had the higher score in 23 of 32 trials, that made from sweet cream with culture added scored higher in 6 trials, while the scores were the same in 3 trials. After 2 months in cold storage,



the ripened cream butter had the higher score in 10 of 35 trials, that made from sweet cream with culture added scored higher in 20 trials and the scores were the same in 5 trials. After 9 months in cold storage, the ripened cream butter had the higher score in 6 of 23 trials, that made from sweet cream with culture added scored higher in 16 trials and the scores were the same in 1 trial.

According to Grimes (37) butter made from sweet cream to which culture had been added without ripening had a higher initial score than butter made from sweet cream.

Lucas *et al.* (65) found that, after 30 days at 0° F., butter made from cream having culture added at churning scored highest, butter made with culture added just before working was second, butter made from cream ripened to 0.30 per cent acid was third, butter made from sweet cream (without culture) was fourth and butter made from cream ripened to 0.45 per cent acid was fifth. The butter made from cream ripened to 0.45 per cent acid declined in average score 1.7 points during the storage while that made from cream having culture added at churning decreased in average score 0.7 of a point. After 3 months in storage the average score of the butter made with addition of culture to the cream at churning was still highest; the butter was excellent and the average score had decreased 1.1 points. Of almost equal quality was the butter having culture added just before working, the average score having decreased only about 0.5 of a point. The sweet cream butter was third and the ripened cream butter was last.

Parsons (89) made butter from split vats of cream, with and without culture. The average scores of the fresh butter showed that culture improved the product. In the individual comparisons butter made with culture scored 0.20, 0.22, 0.27, 0.32 and 0.50 of a point higher than that made without culture. Butter made with culture appeared to be slightly more uniform and had a more pleasing aroma than butter made without culture. A wider difference in score in favor of butter made with culture was found with cream graded to make a 90 to 91 score product than with cream graded to make an 89 score product.

A comparison, by Fabricius and Hammer (83), of addition of culture to pasteurized and cooled, neutralized sour cream at churning and 16 hours before churning indicated that addition at churning consistently gave butter lower in score.

Crossley (21) found it desirable to make butter from sweet cream plus culture, rather than from ripened cream, in order to insure satisfactory keeping qualities during lengthy cold storage. When this procedure is followed, reliance is placed entirely on the flavor of the culture and well-ripened culture is of paramount importance.

Davies (23, 27) stated that the most promising method of using culture consists of employing small quantities of special cultures which are allowed

to produce large amounts of ac.; the culture is added to the cooled cream and the mixture churned without delay.

According to Hunziker (48; p. 363) some buttermakers follow the practice of adding culture to the cream just before churning in order to avoid excessive acidities through cream ripening. Use of 5 to 7 per cent culture usually raises the cream acidity approximately 0.03 to 0.04 per cent. The flavor and aroma of the resulting butter depend solely on the degree to which they are present in the culture. Hunziker concluded that the effect of culture on the butter is slight, and it is questionable whether the practice is economically justifiable.

Jacobsen and Evans (53) obtained a slightly higher general level of scores on butter made with culture than on butter made without culture, when the butter was scored fresh.

**Adding butter culture directly to butter.** When cream with added culture is churned, a portion of the flavor constituents in the culture is lost in the buttermilk. Because of this undesirable partition, there have been various suggestions that culture be worked into the butter. With normal churning procedures the amount of culture that can be added in this way is limited, if the usual composition standards are to be met; with special churning procedures the amount can be increased. Various butter experts originally predicted that working culture into butter would have an undesirable effect on the keeping qualities; in general, this has not been the case but with an unsatisfactory culture there may be dangers along this line.

The Minnesota Agricultural Experiment Station (74) reported that working a small amount of culture into butter at the churn produced butter with a better flavor than that made from ripened cream. Lucas *et al.* (65) concluded that working culture into butter had practically no effect on the curd content or on deterioration. Bouska (10) suggested that the cost of culture can be reduced by working it into the butter.

Hunziker (48; p. 364) stated that working the desired aroma substance directly into butter eliminates much of the added operating expense, avoids the quality-jeopardizing effect of high churning acidities and prevents waste of the flavor principle. He suggested that instead of using water to wet the salt and bring the moisture content to the desired point, several gallons of culture be added to the butter with the salt. Culture so used assists somewhat in freshening the flavor of butter made from unripened cream. It does not increase the acidity of such butter sufficiently to augment the danger of deterioration due to chemical reactions. The keeping qualities of butter so treated are similar to those of butter made from unripened cream without addition of culture. The resulting improvement in flavor, however, is slight; the butter still lacks the full flavor and aroma of ripened cream butter.

*Unsalted Butter*

With unsalted butter intended for table use, a high flavor is very desirable and this necessitates high ripening of the cream, whether it is sweet or neutralized sour cream. Frequently the cream is ripened to 0.45 per cent acid or more, values which often would be disastrous with salted butter because of the greatly accelerating effect of the acid-salt combination on chemical changes in butter. In other cases much lower acidities are employed. Some plants use lower ripening acidities in summer than in winter because during the warmer weather there is more opportunity for development of the culture organisms in the butter itself and this may lead to their overdevelopment, with resulting destruction of flavor constituents (43; p. 115).

High ripening of cream for unsalted butter commonly is carried out at relatively low temperatures, with the temperature often changing during the ripening period as the cream warms or cools from the air of the room. Frequently the temperature of the cream is adjusted to a point which will give the desired acidity during the normal holding period, with the usual inoculation and usual room temperature; in general, the point to which it is reduced is lower in summer than in winter and there may be a shift from day to day as the acidities developed are too high or too low.

Experiments conducted by Jackson (52) indicated that when cream was inoculated with 10 per cent culture, after being neutralized and pasteurized, and ripened 30 minutes at 70° F., the resulting unsalted butter was lower in score than that made from neutralized and pasteurized cream without added culture.

Hunziker (48; p. 363) stated that, with unsalted butter, it is desirable to ripen the cream to a full flavor and aroma. Excellent results are obtained by low-temperature ripening, such as 50° to 55° F., overnight. With this method the cream usually shows a pronounced flavor and aroma the following morning and an acidity of about 0.35 to 0.45 per cent. With ripening at higher temperatures (60° to 70° F.), the aroma character tends to be somewhat more pronounced and the acidity somewhat higher (0.5 to 0.65%).

Hedrick and Hammer (45) studied the effect of ripening cream to various acidities on its  $ac_2$  and  $amc$  contents. Results of ten semi-commercial trials on cream (seven on sweet cream, three on neutralized sour cream) ripened to various acidities at temperatures from 50° to 55° F. showed an increase in  $ac_2$  with an increase in acidity in nine trials (90%) and in  $amc$  in seven trials (70%). Some of the increases were small.  $Ac_2$  decreased in one trial (10%), the drop being from 1.88 to 1.49 ppm. with a change in acidity from 0.37 to 0.49 per cent, and  $amc$  in three trials (30%).

With unsalted butter that is being stored for reconstitution into cream, ice cream mix, etc., small amounts of culture sometimes have been used, the idea being to improve the keeping qualities of the product. The culture has

been added at churning, or some hours before churning when the cream temperature is low, a significant increase in acid being carefully avoided.

#### USE OF SPECIAL CULTURES IN BUTTER MANUFACTURE

Special cultures, instead of the usual butter cultures, occasionally have been employed in butter manufacture, mainly on an experimental basis. They include pure cultures of the normal butter culture organisms and of various closely related species, in some instances the pure cultures being given special treatments intended to intensify flavor development in them, and also butter cultures grown under special conditions. Certain of the special cultures eventually may prove of value in commercial butter manufacture.

Hammer (38) found that addition of *Streptococcus citrovorus* and sterile lactic or citric acid to pasteurized cream resulted in a significant increase in score of the resulting butter; the increase over the control was greater with 0.2 per cent citric acid than with 0.1 per cent. Without added acid the organism gave a slight increase in score. Similar results were obtained with *Streptococcus paracitrovorus*. *S. lactis* gave a better flavor and aroma with sterile citric acid added to the cream than without the acid, but the flavor and aroma of the resulting butter were not as good as those produced by *S. citrovorus* and added citric acid. Addition of sterile citric acid without any inoculation gave a higher score than when *S. lactis* was used without added acid. Later, Hammer (39) noted that considerable flavor and aroma could be produced in butter by growing one of the flavor organisms (*S. citrovorus* or *S. paracitrovorus*) alone in cream that had been carefully pasteurized. A small amount of citric acid added to the cream before inoculation resulted in excellent flavor in the butter.

Bogdanow (8) made butter with *S. lactis*, with *S. cremoris* and with flavor organisms. The best flavor was obtained with *S. lactis*; however, the butter showed the least storage capacity. Butter made with flavor organisms alone had no flavor when fresh and scored lowest.

In the experiments of Maddock (66), butter of exceptionally fine flavor was produced in laboratory tests by use of pure cultures of *S. paracitrovorus*. However, under practical conditions the uncertainties were too great to warrant its general use, the organism being unable to compete successfully with the contaminants encountered in practice.

Fabricius and Hammer (33) compared the use of no culture, regular butter culture and modified butter culture which was prepared as follows: Whole milk was heated to 180° F. for 1 hour, cooled to 70° F. and inoculated with a pure culture of *S. paracitrovorus* in sterile milk. After incubating 24 hours at 70° F., 0.30 per cent sulfuric acid and 0.15 per cent citric acid were added. The culture was incubated another 24 hours at 70° F. and then cooled to 40° F. With sweet cream, modified culture gave butter sig-

nificantly higher in score than regular culture when the butter was fresh and also after holding a few weeks at 28° F. Regular culture gave butter significantly higher in score than no culture when the butter was fresh; after holding at 28° F. the difference was not significant, although the higher score was more often obtained with the culture. Modified culture gave butter significantly higher in score than no culture when the butter was fresh and also after holding at 28° F. Trials involving sour cream showed identical results. Comparisons also were made using no culture, regular culture and regular culture neutralized to 0.30 per cent acid. With sweet cream, regular culture and regular culture neutralized gave butter that did not differ significantly in score when fresh or after cold storage; after holding at 28° F. the butter made with neutralized culture was significantly higher in score. Butter made with regular culture was significantly higher in score than that made without culture when fresh, whereas after holding at 28° F. or after cold storage the difference was not significant. Butter made with regular culture neutralized was significantly higher in score than that made with no culture when fresh and after holding at 28° F., but after cold storage the difference was not significant. Trials involving comparisons of no culture, modified culture and modified culture neutralized were made with sweet cream. Butter made with modified culture was more often higher in score than that made with modified culture neutralized, both after holding at 28° F. and after cold storage; when scored fresh the numbers of higher scores were equal. Differences were significant only with the cold storage butter. Modified culture and also modified culture neutralized gave butter significantly higher in score than no culture in all cases. However, the difference was significant only in case of the fresh butter. Another series of trials involved adding regular butter culture and pasteurized butter culture to sweet cream. The pasteurized culture was made by heating ripened culture to 145° F. for 30 minutes and filtering off the curd. The serum was cooled to 40° F. and used in cream. Butter made with regular culture was more often higher in score than butter made with pasteurized culture when fresh, after holding at 28° F. and after cold storage; differences were significant when the butter was fresh and after holding at 28° F.

Matuszewski *et al.* (69) made laboratory churnings using 5 per cent of a culture of *Streptococcus diacetylactis* to ripen the cream to about 0.5 per cent acid at 68° F. Flavor of the butter was good, but at times was too high, especially after 3 to 5 days at 50° to 59° F. When *S. diacetylactis* was used in combination with *S. cremoris* and *S. citrovorus*, there was an improvement in the butter compared to butter made with regular culture.

Comparisons were made by Brewer *et al.* (11, 12) to determine the quality of butter produced by use of aerated-pressure butter culture. These usually were prepared by adding 2 per cent active culture to pasteurized skimmilk, although in some cases whole milk was used to prevent excessive

foaming during aeration. After inoculation the milk was divided, one portion being ripened with aeration under 60 pounds pressure, while the other was ripened as usual to serve as a control. The ripened cultures were cooled and then added to divided lots of pasteurized sweet cream. After appropriate holding, the mixtures were churned. Results showed that aerated-pressure culture gave butter scoring 0.15 to 1 point higher than that made with regular culture; with the aerated-pressure culture the flavor was higher with both sweet and sour cream butter. In trials in which 5 per cent regular culture and only 2 per cent aerated-pressure culture were added to cream, the butter made with the aerated-pressure culture regularly had the higher flavor.

Khubchandani (55) found that use of *S. citrovorus* cultures, treated with citric acid or sodium citrate 24 hours prior to inoculation into cream, gave better flavor and aroma in butter than use of untreated cultures. The keeping qualities of such butter were not affected by the citric acid or sodium citrate.

Davies (27) attempted to produce butter of average flavor and low acidity by use of pure cultures of *Betacoccus cremoris* and *S. paracitrovorus*. The cultures were employed successfully in the laboratory but became contaminated in plants and finally were essentially regular butter cultures.

#### SURVIVAL AND GROWTH OF BUTTER CULTURE ORGANISMS IN BUTTER

Rapid growth of the butter culture organisms in milk, cream, etc., at favorable temperatures suggests that they would grow rapidly in butter. However, in the case of salted butter the added salt is an important inhibiting factor and with both salted and unsalted butter the low holding temperatures often used and the small amounts of milk solids-not-fat present also are of significance in limiting the growth. Butter culture organisms sometimes survive for extended periods in butter.

#### *Salted Butter*

Sayer *et al.* (109) found a rather regular decrease in the numbers of lactic acid bacteria in butter during storage at 41° F. The decrease was related to the concentration of salt in the butter, higher concentrations giving greater decreases. Apparently the salt content was not the only reason for the decrease in numbers.

Washburn and Dahlberg (133) reported that *S. lactis* withstood salt and low storage temperatures better than any other organism in butter. It was the only species appearing regularly in all lots of butter. After 304 days in storage, every lot of salted butter contained essentially only *S. lactis*. Brown *et al.* (15) noted that lactic acid bacteria persisted in butter for as long as 275 days (in one case 426 days). According to the authors salt and low temperatures may cause such a slow rate of metabolism that relatively

little acid is produced and life of the cell is prolonged. Butter in storage showed a gradual decrease in the amount of lactose and a gradual increase in acidity.

Grimes (37) found that butter made from cream to which culture had been added, either without ripening or with ripening to various acidities, retained per ml. from 0.5 to 2.0 per cent of the culture organisms per ml. of the cream-culture mixture. The flora of the butter consisted of *S. lactis* and *S. paracitrovorus*, *S. lactis* making up 70 to 100 per cent of it. After 6 months at  $-6^{\circ}$  F. over 98 per cent of the bacteria had died. After cold storage, sweet cream butter often had a higher bacterial count than butter made from cream to which culture had been added. In general, it appeared that the normal flora of pasteurized cream found an acid environment unfavorable; also, utilization of lactose by *S. lactis* and *S. paracitrovorus* was thought to be a factor. The flora of the butter when taken out of cold storage consisted chiefly of *S. lactis* and acid-forming, non-coagulating species. *S. paracitrovorus* usually was present. *S. lactis* was not found in pasteurized cream butter made without culture when first manufactured but was found after cold storage.

It was noted by Parfitt (88) that salted butter made with culture showed less variation in pH during storage than butter made without culture.

In the trials of Nelson and Hammer (81) butter was churned under both laboratory and commercial conditions. With the laboratory butter, one series of trials involved butter made from cream of poor quality so that the microscopic count of the fresh control butter was high, considerable numbers of both streptococci and other organisms being present. During holding at  $69.8^{\circ}$  F. the numbers of organisms in the control butter showed comparatively little change. With three of the organisms (butter culture types) used in the cream, the butter showed fluctuations in the numbers of streptococci during the holding in both the plate and microscopic counts, but there were no definite trends evident. With the fourth culture organism used, each method of counting indicated a definite increase in the numbers of streptococci. Throughout the examinations the microscopic counts regularly were much higher than the plate counts with both the streptococci and the other organisms, and the ratio between the two was extremely variable. Another series of trials involved cream of very good quality and the microscopic and plate counts on the fresh control butter were low and did not show significant changes during the holding. With the butter made from cream inoculated with culture organisms, there was a general tendency for a decrease in the numbers of streptococci with both methods of counting, and the decrease definitely was greater on a percentage basis with the plate method than with the microscopic method. With butter made under commercial conditions, the general change in the numbers of streptococci, as shown by the microscopic counts, was a decrease that commonly was exten-

sive; with certain samples it was very regular while with others some fluctuation occurred. With several samples there was a suggestion of a small increase in the numbers of streptococci after 1 day, but the evidence was not at all convincing. Plate counts showed the same general changes in numbers of organisms. The streptococci appeared to be greatly autolyzed and the chains seemed to be shorter after 7 days than after 2 days.

### *Unsalted Butter*

Rahn *et al.* (100) noted that all lots of butter made without salt and stored at 42.8° F. showed a very high increase in acidity, compared to lots of salted butter. When held at 21.2° F. the unsalted butter showed a slightly higher increase in acidity than the salted butter.

Nelson and Hammer (81) found a definite development of the butter culture streptococci in unsalted butter held at a favorable growth temperature. It was evident from both plate and microscopic counts on butter prepared in the laboratory, using a pure culture of *S. lactis* or one of the flavor organisms, and also on butter prepared under commercial conditions with butter culture. Growth resulted in the presence of long chains of streptococci and these were striking evidence of multiplication since they were not found in the salted butter or in butter (salted or unsalted) made without addition of organisms to the pasteurized cream. In the unsalted butter there also was extensive growth of organisms other than streptococci, as shown by both plate and microscopic counts.

Hammer (42) noted that in unsalted butter made with butter culture and held at 69.8° F., acidity of the serum increased more rapidly and reached higher final values in underworked butter than in thoroughly worked butter; the relationship was evident with either a small or a large inoculation of the cream.

Effect of reworking on changes in the pH of serum obtained from unsalted butter made with culture was investigated by Long and Hammer (64); pH determinations were made at once, at reworking and at different periods following the reworking. In the laboratory studies, some trials involved underworked and moderately worked butter, while others involved moderately worked and thoroughly worked butter. The more the control butter was worked, the slower were the decreases in pH of the serum. Reworking regularly resulted in a more rapid decrease in pH of the serum, and this was evident at each examination. In two trials on underworked and moderately worked butter the differences were considerable, while in one trial, also on underworked and moderately worked butter, the differences were less extensive. In one trial the control portion of the thoroughly worked butter showed relatively little decrease in pH of the serum throughout the holding period, while the reworked portion showed considerable change; the final pH value of the serum of the reworked portion was rela-



tively high compared to those of underworked and moderately worked butter similarly treated, suggesting that thorough working of the original butter may tend to counteract the effect of reworking. Butter churned under commercial conditions with a relatively large amount of culture in the cream showed the same general decrease in pH of the serum as butter made under laboratory conditions.

#### SOURCES OF NATURAL BUTTER FLAVOR

With butter made from raw cream allowed to sour spontaneously, the flavor is largely determined by the changes that go on during the holding of the cream, particularly those due to micro-organisms. When the fermentation is favorable the flavor of the butter is desirable, and when it is objectionable the flavor of the butter is undesirable. A desirable flavor is due to action of certain types of bacteria which include the species normally present in butter cultures and perhaps others as well. Actually, butter cultures were developed with the idea of duplicating the flavor of the best of the butter made from naturally soured cream.

Butter made without culture from cream in which there has been no appreciable bacterial action has a flavor which is due to the milk constituents, particularly the fat, plus flavoring materials absorbed or added during production and handling of the milk, cream or butter. The feed, season, stage of lactation of the cows and various other factors may influence the composition of the milk and the flavor of butter made from it. Even slight bacterial action in the milk or cream may modify the flavor. For many consumers, proper use of butter culture definitely improves the flavor.

Conn (18) noted that the flavor of butter is not due to butterfat but to certain volatile products present in small quantities. The odors produced by various bacteria plainly indicated that volatile substances were being produced during the ripening of the cream as a result of the fermentation. It was impossible to notice the odors produced by the growth of various species of bacteria without being convinced that they had something to do with the superior flavor of butter made from ripened cream.

Ruehle (108) indicated that the flavor of butter is dependent on the type of fermentation which occurs in the cream previous to churning. Hammer (41) stated that the desirable flavor and aroma of butter come from two main sources. With butter made from cream in which there has been no development of micro-organisms, the desirable flavor comes from the milk constituents present, particularly the fat, while with butter in whose manufacture culture has been used, the desirable flavor comes from the milk constituents and also from the products formed by the culture organisms. Ruehe (106) suggested that the flavor and aroma of butter are the grand total of the natural flavor of fat as it is normally produced by the cow, plus the flavors of the ingredients added during manufacture, plus the flavors

and odors absorbed during handling and storage, plus flavors caused by activity of micro-organisms, plus flavors which result from chemical changes in the product. Neuburger (82) noted that the amount of butter aroma depends on the season of the year and feed of the cows.

Another general comment (103) stated that the flavor of butter is due primarily to the natural flavor of the cream from which it is churned. Butter made from cream in which there has been no bacterial fermentation has a characteristic flavor, agreeable but mild. The nature of the flavor is dependent to some extent on the feed and may be affected unfavorably by ingestion of highly-flavored feeds of various kinds. Fat is a solvent for many flavor and aroma-producing substances and the odors and flavors to which milk or cream may be exposed, as well as those formed in the serum by bacteria, yeasts or molds, may be carried into the butter.

Results obtained by Sherwood and Hammer (113) suggested that seasonal variations in flavor and aroma of butter are not related to differences in the citric acid content of the milk or cream. Experiments conducted by Steuart (118) indicated that the composition of the fat is an important factor in the production of aroma.

#### EFFECT OF BUTTER CULTURE ON FLAVOR OF BUTTER

Following the introduction of butter cultures, their use spread rapidly, which definitely indicates they are of value in improving the flavor of butter. Many plants continue to use culture even when there are serious difficulties in the procurement of milk for it. In others, use of culture has been discontinued, the idea often being that culture increases the cost of butter manufacture and that the flavor improvement obtained is not reflected in a price increase.

Over the years there have been various reports covering the effect of culture on the flavor of butter. A number of these are reviewed in the consideration of the methods of using butter cultures in the manufacture of butter but there are others of significance.

Bouska (10) indicated that if butter made without culture lacks aroma, it is handicapped in maintaining its advantage as a spread for bread in competition with substitutes.

Wilster (138) stated that many experienced creamerymen and butter dealers believe the use of fine-flavored culture in first-grade, neutralized sour cream and in sweet cream results in butter which scores higher in flavor than that made without culture. When butter is sent to a scoring contest, culture is used with the majority of the churnings. The average score of butter made with culture usually is higher than that of butter made without culture. In a cold storage contest of the National Creamery Buttermakers' Association at St. Paul, Minnesota, in 1924, culture butter averaged 0.93 of a point higher in score when fresh than butter made without culture. In a

similar contest at Cleveland, Ohio, in 1928, culture butter averaged 0.65 of a point higher in score when fresh than butter made without culture. At the Pacific International Dairy Products Show at Portland, Oregon, in 1931, butter made with culture averaged 1.40 points higher in score than butter made without culture. At the Oregon Butter and Ice Cream Makers' Annual Convention at Corvallis, Oregon, in 1932, butter made with culture averaged 0.67 of a point higher in score than butter made without culture. At the Pacific International Dairy Products Show at Portland, Oregon, in 1935, butter made with culture averaged 0.80 of a point higher in score than butter made without culture.

Fabricius (31) listed the arguments in favor of use of butter culture as follows: (a) Overcomes heated flavor, (b) decreases lime and burnt neutralizer flavor, (c) helps to overcome some feed defects, (d) may help to overcome tallowy flavors and (e) in unsalted butter its use decreases danger of cheesy, putrid and surface taint flavors. The arguments against the use of butter culture were listed as follows: (a) Cost, (b) improper use may cause oily and fishy flavors, (c) accentuates some feed flavors, (d) good culture is difficult to make and (e) price differential on the market does not justify its use.

Hoecker and Hammer (47) found that butter made with butter culture, *S. citrovorus*, *S. paracitrovorus* or *Streptococcus citrophilus*, usually contained relatively large amounts of  $ac_2$  and amc and commonly placed high in a series of churnings. However, butter containing exceptionally large amounts of  $ac_2$  and amc sometimes placed low and was criticized as being coarse, sour, oily or containing some other objectionable flavor. Butter made with *S. diacetylactis* usually contained large amounts of  $ac_2$  and amc but often was placed low because of off flavors present. Butter made without culture or with *Streptococcus aromaticus* contained only small amounts of  $ac_2$  and amc and usually placed low.

#### EFFECT OF BUTTER CULTURE ON KEEPING QUALITIES OF BUTTER

Consideration of the effect of butter culture on the keeping qualities of butter involves two distinct relationships. The first is the effect on the general score and concerns largely chemical changes in the butter, although with some lots of butter bacterial changes also are of significance, while the second is the effect in preventing certain bacterial defects of butter.

##### *On General Score*

Use of culture in butter manufacture gives the product more flavor but, because of the influence of serum acidity on chemical changes, it may result in serious deterioration. In salted butter a high flavor due to culture and good keeping qualities over long periods are more or less incompatible. Present methods of using culture are intended to give as much flavor im-

provement as possible without seriously affecting keeping qualities, the period the butter is to be held being considered.

It should be recognized that serum acidity is only one of the factors involved in chemical deterioration in butter and that some of the changes attributed to acid may have been greatly influenced by some other factor, such as contamination with copper. A serum acidity which does not result in significant deterioration when the copper content of the butter is low may be very serious when the copper content is high (50).

The early studies of Rogers and Gray (104) indicated that butter made from pasteurized cream with culture added does not have as good keeping qualities as that made without culture. A report from the Ontario Agricultural College (83) suggested that butter of improved quality may be made from high acid cream by neutralization and addition of good culture. A low churning acidity was more favorable to good keeping qualities in butter than a high churning acidity.

Mortensen (80) found that butter made from sweet cream with culture added scored higher after 2 months at 0° F. than butter made without culture, while after 9 months it scored lower. Butter made from sweet cream with culture added scored higher after 2 months and also after 9 months than butter made from ripened cream.

White *et al.* (134) stated that the improvement in flavor of fresh butter resulting from addition of culture to the cream usually is lost when the butter is placed in cold storage.

According to Lucas *et al.* (65) addition of a moderate amount of culture to cream before churning, or to the butter at the time of working, caused the butter to score higher after 3 months at 0° F. than if none had been used. Whether the aroma due to the culture covered the slight off flavors that normally develop in sweet cream butter during storage, or whether some inhibitory action took place when culture was used, the authors were unable to say. Ripened cream butter deteriorated more rapidly during storage than butter from sweet cream, butter from sweet cream with culture added just before churning or butter to which culture was added at the time of working. No relationship was found between use of culture and the iodine number of butterfat from the butter. The increase in acidity of cream obtained by using culture seemed to cause a slight increase in the acid number of the fat.

Results obtained by Hammer and Jensen (44) indicated no evident relationship between acidity of butter culture and quality and keeping qualities of butter made with it from sweet cream pasteurized at 145° F. for 30 minutes. When fresh, and also after storage, butter made with high acid culture and butter made with low acid culture ranked high or low approximately the same number of times. The small variations noted in the deterioration of the butter in storage seemed to be due to much or little

deterioration in one or two lots and did not regularly follow differences in acidities of the cultures. There was no evident relationship between acidity of the culture and quality and keeping qualities of butter made from neutralized sour cream pasteurized at 145° F. for 30 minutes. A somewhat greater variation was noted in the deterioration during storage than with butter made from sweet cream, and the difference was most conspicuous in the butter made with high acid culture; however, the variation did not follow the acidities of the cultures. Acidity of the culture would not be expected to have a great effect on the quality of fresh butter made by adding 10 per cent culture to pasteurized cream without ripening. With this method of manufacture, the flavor and aroma of the butter are directly influenced by the flavor and aroma of the culture, so that with cultures of good quality variations in acidities would be comparatively unimportant. Neither would large variations in the deterioration of the butter be expected to follow variations in acidities of the cultures because the great dilution of the added culture with cream of a much lower acidity results in only very small differences in the acidities of the butter serum. Butter made from neutralized sour cream showed a somewhat greater variation in score decreases during storage than butter made from sweet cream; this may have been due to differences in the quality of the cream.

Parsons (89) found little difference in keeping qualities of butter made with and without culture.

Results of Overman (86, 87) showed that the lots of butter which held their score best were churned from fresh cream separated from fresh, sweet milk; they were not salted and were salable after about 2 years in storage. Butter made from pasteurized sweet cream after ripening with culture and then partially neutralizing was correspondingly better than butter from the same lots of cream after spontaneous souring and neutralizing to the same acidity. Butter made from cream ripened with culture to 0.44 per cent acid and churned without neutralization scored higher and held its score better in storage than butter from the same lots of cream after ripening with culture to 0.61 per cent acid and churning without neutralization; the latter was correspondingly better than butter from the same lots of cream after ripening with culture to 0.82 per cent acid and churning without neutralization.

Bogdanow (8) reported that ripening cream with culture decreases the storage capacity of the butter.

Templeton (121) found that the storage qualities of butter made from cream in which culture, containing added citric acid or sodium citrate, had been used were very good. This was indicated by the fact that numerous samples which had been in storage for 6 weeks received higher scores than when fresh.

Wilster (138) noted that in the scoring contest conducted by the National Creamery Buttermakers' Association at St. Paul, Minnesota, in 1924, butter

made with culture scored 0.87 of a point higher after 4 months in storage than butter made without culture. In a similar contest at Cleveland, Ohio, in 1928, butter made with culture scored 0.61 of a point higher after 4 months in storage than butter made without culture. Wilster stated that approximately 100,000 pounds of butter are made annually in the Oregon State College creamery. Culture always is added to the cream and the cream is ripened to a serum acidity of 0.42 to 0.44 per cent. About 15,000 to 20,000 pounds of butter are stored during the summer for consumption in the fall. No objectionable flavors had developed in the butter made during the 6 years in which culture had been employed. There was no marked decrease in the score of butter made with or without culture during 1 month at 35° to 45° F. However, a decrease in score was observed when butter was stored 6 months at 0° to 10° F. The average decrease in score of the butter made without culture was 0.57 of a point. The average decreases for butter made with culture were 1.15 points when the cream was ripened, 0.74 of a point when culture was added and the cream held cold 16 hours previous to churning and 0.95 of a point when culture was added and the cream churned immediately; the average scores after 6 months, compared to that of butter made without culture, were 0.30, 0.66 and -0.06 of a point in favor of the respective methods of using culture. In one series of churnings, butter was made from 93 lots of cream, culture being used with 59 and no culture with 34. When the butter was stored 1 month at 35° to 45° F. and 6 months at 0° to 10° F., the average scores of the butter made with culture were higher than those of the butter made without culture. Chief comments of the judges on the butter made with culture were "creamy flavor," "sweet and clean," "fine aroma" and "fine flavor"; those on the butter made without culture were "flat," "lacking character," "insipid" and "tallowy." (See also 96.)

The results of Wiley (135) indicated that the presence of culture organisms in butter favored oxidation of the fat at storage temperatures. The oxidation that occurred in the presence of culture organisms, even when the acid produced in the culture was neutralized before churning, demonstrated the presence of some fat-oxidizing system in the ripened cream. This was believed due to some product of bacterial metabolism acting as a pro-oxidant; if this is the case, the product is not  $\text{amc}$  or  $\text{ac}_2$ . Wiley considered the results may be explained by assuming that the lactic acid streptococci of the culture contain a fat-oxidizing enzyme which is most active at low pH and high salt concentration. Several mixed cultures (*S. cremoris* and betacocci) and a single strain of *S. cremoris* all increased oxidation, as compared to simple acidification of the cream.

According to Hunziker (48; p. 357) there are certain conditions of butter manufacture which permit controlled ripening without jeopardizing keeping qualities, and there are still other conditions of manufacture and distribu-

tion under which ripening to a full aroma and flavor is definitely beneficial to keeping qualities. The extent to which cream may be ripened and still have the butter reach the consumer without objectionable flavor deterioration due to chemical changes was considered to be influenced by such factors as: (a) Condition and quality of the original cream, (b) temperature at which butter is held between churn and consumer, (c) whether butter is intended for fresh consumption or for cold storage, (d) whether the cream is used for salted or unsalted butter and (e) salt concentration in salted butter. Hunziker (48; p. 353) also stated that cream ripening does not improve the chemical stability of butter and that, under average commercial conditions of manufacture, the ripening of cream to a full flavor and aroma shortens the life of salted butter. Usual flavor defects which develop with age in salted butter made from fully ripened cream were said to be oily-metallic, fishy and sometimes tallowy flavors. Salted butter made from sweet, unripened cream or from sour cream neutralized and pasteurized was said to keep better, from the standpoint of flavor deterioration due to chemical causes, than salted butter made from the same cream ripened to full flavor and aroma.

Jacobsen and Evans (53) found that butter made from first-grade cream without culture had better keeping qualities than butter made from first-grade cream with culture; the butter was scored after 3 and 5 months at 32° to 36° F.

#### *In Preventing Certain Bacterial Defects*

Growth of various bacteria responsible for development of specific defects in butter apparently often is delayed or prevented by use of butter culture in the cream. The inhibitory effect apparently is due not only to the products formed by the culture organisms but also to the presence of the organisms themselves.

Orla-Jensen (84) inoculated butter with *Pseudomonas fluorescens* and *S. lactis*. He noted that the lactic acid formed by *S. lactis* inhibited fat hydrolysis; there was little free volatile acid produced and the odor and taste of the butter were not especially objectionable.

Rogers (102) stated that during ripening of cream the lactose is partially fermented to lactic and similar acids which protect the butter from fermentation by less acid-tolerant organisms. The putrefactive bacteria, which often attack the curd of butter, usually were checked by the acid produced during cream ripening.

Results obtained by Mazé (70) indicated that micro-organisms causing deterioration in butter largely attack the casein and lactose. They were found to be retarded by lactic acid. Addition of lactic acid to butter at the rate of 0.5 to 1.0 g. per l. was suggested as a control measure. Lactic acid was not believed to completely prevent growth.

Shutt (114) reported that churning cream at an acidity of not less than 0.35 per cent was advantageous in preventing defects caused by *Ps. fluorescens* since it grows only feebly at a pH of 6.6. He was of the opinion that surface taint could be caused by *Ps. fluorescens*, but that it occurred only in sweet cream or neutralized cream butter and never in sour cream butter.

Virtanen (127) noted that water types of micro-organisms, which frequently cause cheesy and putrid defects in butter, are inhibited by the acid of sour cream butter.

Derby and Hammer (30) made butter from pasteurized cream inoculated with surface taint butter and studied the influence of salt and butter culture on production of the defect. Unsalted and low salted butter made without the culture developed surface taint in 2 days at 70° F., but medium salted butter made without the culture, unsalted butter made with the culture and salted butter made with the culture was normal after 7 days. Claydon and Hammer (17) added *Pseudomonas putrefaciens* to pasteurized cream and churned the cream with various amounts of butter culture added. None of the unsalted or salted butter made with the culture developed the putrid defect in 6 days at 70° F. but that made without the culture became markedly putrid. Five per cent culture inhibited the defect as effectively as 12 per cent. Results indicated that butter culture had an inhibiting effect on development of the putrid defect in unsalted butter when *Ps. putrefaciens* gained entrance to the butter either from cream or from wash water.

According to Knudsen (57) ripening cream with butter culture protects the resulting butter from attack by putrefactive bacteria which may get into it from water, but which cannot develop because the reaction is acid; ripening the cream with culture promotes development of certain defects, particularly in storage. Minster (75) considered the presence of a few lactic acid bacteria in unsalted butter as beneficial, since they tended to keep down growth of objectionable species.

In the trials of Hussong *et al.* (49) butter made from pasteurized cream inoculated with *Pseudomonas fragi* developed a rancid flavor somewhat less rapidly when 10 per cent butter culture was added to the cream just before churning than when the butter was made without culture.

St. von Nyiredy (119) reported that in butter made from ripened cream, coliform, proteolytic and *Ps. fluorescens* organisms showed practically no growth and decreased rapidly, while they increased markedly in sweet cream butter. The ripened cream butter had a pH of 4.6 to 4.8 and the sweet cream butter a pH of 6.4 to 6.8. It was concluded that failure of these groups to grow in ripened cream butter was due to the low pH.

Hunziker (48; p. 295) stated that efficient pasteurization of the cream, preferably followed by proper ripening with good culture, not only retards the general age deterioration which is characteristic of all unsalted butter but prevents the early appearance of the more serious flavor defects result-



ing from the presence in cream of specific flavor-damaging organisms. When unsalted butter was made from cream ripened to full flavor and aroma, bacterial deterioration was very noticeably retarded by the predominance of lactic acid bacteria and the relatively high acidity. These agencies assisted in checking the organisms which, if present, caused bacterial flavor defects in unsalted butter (48; p. 360). In comments on cheesy flavor, Hunziker (48; p. 668) noted that resistance of unsalted butter to bacterial age deterioration and to development of cheesy flavor is materially improved by ripening the cream with a good culture to a pronounced culture character and a reasonably high churning acidity (preferably about 0.35 to 0.45 per cent). It appeared that the great predominance of culture bacteria and the products of cream ripening are more or less antagonistic to the organisms responsible for cheesy flavor and definitely assist in holding them in check.

Fouts (35) studied the effect of butter culture and lactic acid on hydrolysis of fat in cream by pure cultures of lipolytic bacteria. Butter culture added to the cream definitely inhibited *Achromobacter lipolyticum*, *Alcaligenes lipolyticus* and *Ps. fluorescens*. When lactic acid was added to the cream used, all the organisms grew, even with enough acid to give a titrable acidity of 1 per cent.

Results obtained by Itzerott (51) showed that cream acidities below 0.15 per cent had little effect on the period required for development of the putrid defect in butter. Above 0.15 per cent, however, the acidity appeared to have a retarding influence. High acidities and low temperatures definitely inhibited the defect. Unsalted butter made from cream with 0.20 per cent acid and inoculated with rabbito organisms showed no evidence of the defect when held 3 weeks at 40° to 55° F. At higher temperatures (80° to 90° F.) the taint developed, even with a high acidity.

Pont (95) indicated that, within the range of safe limits from the standpoint of chemical changes in butter, high acidities aided in minimizing the putrid defect of butter.

Effect of butter culture on the ability of 52 fluorescent bacteria to produce defects in experimental butter was studied by Garrison and Hammer (36). Addition of 10 per cent culture to the cream prevented development of off flavors in unsalted butter by 16 of the cultures and in salted butter by 27 of them.

#### RELATIONSHIP OF SPECIFIC CHEMICAL COMPOUNDS TO DESIRABLE FLAVOR OF BUTTER

The desirable flavor produced in butter through use of butter culture is dependent on various compounds, some of which still may not have been identified.  $Ac_2$  apparently is the most important of these but others, especially volatile acids, also are of significance.  $Ac_2$  and volatile acid are

produced in butter culture, and also in ripening cream, primarily from citric acid by the normal flavor organisms while lactic acid is produced primarily from lactose by *S. lactis* (43; p. 84).

### *Volatile Acids*

In 1890, Conn (19) stated that it usually is believed the flavor of butter is due to certain volatile fatty acids formed during the ripening of cream. The acids considered to be important were butyric, caproic, caprylic and capric.

Ferris *et al.* (34) determined the volatile acids in 14 lots of commercial sweet cream butter scoring 93 to 94. The values, expressed as ml. N/10 acid per 100 g., ranged from 0.2 to 0.4 by steam distillation and from 0.5 to 0.8 by direct distillation. The average score of the lots after storage 5 to 6 months at 22° F. was about 1 point lower than when they were fresh and the volatile acid values were about twice as large. After 6 to 7 months, the butter was removed from storage and kept 2 weeks at about 59° F. This caused a decided drop in score but no significant increase in volatile acid. More volatile acid was found in butter made from clean acid cream than in butter made from sweet cream.

Lind (63) determined the volatile acidity of butter by adding 150 ml. water and 15 ml. N/1 sulfuric acid to 100 g. butter and distilling with steam. From values obtained on skimmed milk, pasteurized cream and butter, the author concluded that butter dissolves a part of the volatile acid formed by the bacteria of the culture employed for acidification of the cream.

Results of Steuart (118) indicated that when full-flavored butter is distilled with steam, free butyric acid can be identified in the distillate as well as a neutral compound yielding butyric acid on saponification. Use in margarine of cultures producing butyric acid or addition to margarine of ethyl butyrate or butyric acid gave unsatisfactory results. Butyric acid itself was too fugitive, and a search was made for a parent substance which would continuously yield traces of the acid. In experiments with margarine prepared with skimmilk properly soured by lactic acid and aroma bacteria, it was found that a small addition of tri-butyryl caused the mass to be gradually pervaded by a pleasant butter aroma. Unfortunately, the flavor was masked by bitterness of the tri-butyryl. This compound was obtained from various sources and was synthesized, fractionated and refined, but in no instance was the bitterness avoided. Glycol di-butyryl produced a similar aroma in margarine but also had an inherent bad flavor. Mixed butyric tri-glycerides were then prepared and the flavor of these indicated that the butyric acid in butterfat must be present as a mono-butyric tri-glyceride, with butyric acid in the beta position. The bitterness of tri-butyryl is due to butyric acid in the alpha position. Coconut oil was butyrylated by heating with butyric acid at 320° F. After treatment for 2

hours the neutralized fat gave Gilmour butyric numbers equivalent to 3 per cent butter and after treatment for 36 hours the numbers were equivalent to 116 per cent. Fat also could be butyrised by heating it with tributyrin in the presence of a suitable catalyst, such as 1 per cent stannous hydroxide, at 392° to 482° F., the mixture being agitated by allowing an inert gas to bubble through it. Coconut fat containing from 5 to 15 per cent tri-butylin was free of bitterness after 2.5 to 7 hours; while arachis oil with 25 per cent tri-butylin required treatment for 11 hours.

*Acetylmethylcarbinol and Diacetyl*

van Niel *et al.* (126) studied the relationship of  $\text{amc}$  and  $\text{ac}_2$  to the aroma of butter. Analyses on butter, using 50 g. portions, showed that lots having a typical aroma gave an  $\text{amc}$  reaction while lots lacking such an aroma did not. A series of lots that had been given definite scores on aroma showed a rather close correlation between a high aroma and the presence of  $\text{amc}$ . The authors further observed that a dilute aqueous solution of  $\text{amc}$  had an odor suggesting butter and that  $\text{ac}_2$  also had such an odor. However, properly purified  $\text{amc}$  was odorless. The results suggested that  $\text{ac}_2$  is responsible for the flavor of butter. When 0.0002 to 0.0004 per cent  $\text{ac}_2$  was added to butter lacking aroma, an unmistakable aroma appeared. The authors concluded that  $\text{ac}_2$  is either responsible for the aroma of butter or is the principal component of the aroma material.

The conclusion of Testoni and Ciusa (123) was that the presence of  $\text{ac}_2$  in butter is accidental and that  $\text{ac}_2$  cannot be considered one of the aromatic principles of butter.

Waser and Mohler (132) noted that even after purification, and in very dilute solution,  $\text{ac}_2$  displayed the characteristic odor of butter. Tapernaux (120) stated that the artificial perfumes with an  $\text{ac}_2$  base have given good results in creating the artificial aroma of butter in various fatty materials. Development of aroma in natural butter was believed due to the formation of  $\text{ac}_2$  by fermentation.  $\text{Ac}_2$  was not considered the only substance responsible for development of the natural aroma, but it was believed to be the fundamental base of butter aroma since small amounts possessed a very penetrating odor.

Kay (54) reported that the flavor, and particularly the aroma, of butter is due largely to the presence of  $\text{ac}_2$ . In commenting on this idea, Cox (54) stated that he had tested many samples of butter but had never found  $\text{ac}_2$ . The compound was not considered present in natural butter.

According to Hammer (40) the odor of  $\text{ac}_2$  in high concentrations is pungent and not at all pleasing; however, in low concentrations the aroma is like the aroma of fine butter made from clean-flavored sweet cream with use of culture. Results obtained by Barnicoat (6) indicated that a fraction of a part of  $\text{ac}_2$  per million imparts a rich aroma to butter made from un-

ripened cream. Examination of mild-flavored butter showed amc to be present.

Pien *et al.* (91) stated they had good reason to assume that  $ac_2$  is not the cause of aroma in butter even though dilute aqueous solutions have an odor resembling butter and addition of  $ac_2$  to butter without aroma gives an unquestionable aroma.

Virtanen and Tarnanen (129) considered  $ac_2$  to be the substance responsible for the flavor of butter. Davies (23) reported that the compound responsible for most of the flavor of butter is the diketone,  $ac_2$ , which can be produced synthetically or biologically. Butterfat itself was believed to contribute to the flavor, as well as traces of esters, such as ethyl butyrate, caproate and lactate, the curd and the salt. According to Slatter (116) the desirable flavor and aroma of butter are due chiefly to the fat, volatile acids and  $ac_2$ .

Analyses by Pien *et al.* (92) showed that butter high in  $ac_2$  had a very desirable flavor and aroma. However, many samples low in  $ac_2$  also were excellent in flavor and aroma. Results indicated that constituents other than  $ac_2$  are responsible for the desirable flavor and aroma of butter. Pien (90) noted that when  $ac_2$  was absent in butter, the aroma of the butter was weak or there was no aroma.

Mazé (71) stated that the normal lactic acid organisms produce neither amc nor  $ac_2$  and the presence of these compounds in butter proves the existence of an incidental and noxious fermentation.  $Ac_2$  was believed to occur in the blood and, because of this, traces of it were thought to be carried into the milk.

Results of Davies (25, 26) indicated that the flavor and aroma of ripened cream can be closely simulated by working synthetic  $ac_2$  into flavorless butter. However, this procedure gives a somewhat harsh flavor which may be due to the fact that the added  $ac_2$  does not have the same opportunity to distribute itself between the fat and water phases of butter as the  $ac_2$  slowly formed during cream ripening, or it may be due to modification of the effects of  $ac_2$  by other flavoring compounds, such as esters and other volatile organic compounds, which are produced in traces in the cream. The author indicated it is generally agreed that the compound responsible for the aroma and flavor of butter is  $ac_2$  and that it is formed together with somewhat larger amounts of amc.

Experiments by Makar'in (68), designed to test previous theories regarding formation of aroma in butter, indicated that the most important part is played by amc and  $ac_2$ .

Pont (94) noted that it is generally agreed the intensity of flavor and aroma of butter varies with the  $ac_2$  content. Where full development of the butter culture in the cream was permitted, it appeared that the flavoring element existed mainly in the form of amc due to the low oxidation-reduction

potential of the lactic acid fermentation. Besides  $ac_2$ , other flavor contributants were butterfat and traces of esters like ethyl butyrate, caprate and lactate.

According to Langton (59)  $ac_2$  is the cause of the aroma of butter. Davies (27) stated that addition of  $ac_2$  to fats in amounts equal to those found in natural butter gives a harsh, artificial flavor. He suggested that when synthetic  $ac_2$  is added, only about one-tenth of the amount found in butter of equal flavor should be employed. The main argument in favor of the use of synthetic preparations was that non-flavored butter intended for long keeping could be made from sweet cream and the flavor added without detriment to keeping qualities.

#### *Homologs of Diacetyl*

According to Waser and Mohler (132) the next higher ketone homolog of  $ac_2$ , triketopentone ( $CH_3 \cdot CO \cdot CO \cdot CO \cdot CH_3$ ), displays a more intense odor than  $ac_2$ . It possesses a penetrating and adhering aroma of ginger bread spice.

Prill *et al.* (97) noted that acetylpropionyl, acetyl-*n*-butyryl, acetyl-*iso*-butyryl, dipropionyl, acetyl-*n*-caproyl and di-*n*-butyryl resembled  $ac_2$  in odor and certain other properties, but methylglyoxal and other alpha keto-aldehydes, as well as the alicyclic and aromatic diketones, differed distinctly from it in odor and also other respects. The various compounds showed similar behaviors in many of the reactions used for determination of  $ac_2$ . Methylglyoxal and glyoxal, however, could be distinguished from alpha diketones by means of the alpha-methylindole test. Certain homologs of  $ac_2$  (acetylpropionyl and dipropionyl), as well as  $ac_2$ , could be determined colorimetrically as diammino-ferrous derivatives of their dioximes. When acetylpropionyl or dipropionyl was worked into salted butter, the effect on the flavor definitely suggested the effect of  $ac_2$  but was less than with an equivalent amount of  $ac_2$  and was less with dipropionyl than with acetylpropionyl. In butter, acetylpropionyl or dipropionyl contents, comparable to the  $ac_2$  contents developed through use of cultures, were well retained over a considerable period at various temperatures. Distillates from ordinary butter cultures gave no evidence of the presence of higher homologs of  $ac_2$  or  $ame$ ; methylglyoxal also was absent.

#### *Miscellaneous*

Aschan (4) concluded that large quantities of butter are required to obtain the active constituents causing the aroma of butter and that a more profitable attack would be to synthesize the mono- and di-glycerides of the fatty acids which affect the senses of taste and smell.

Tapernaux (120) stated that numerous chemical products have been recommended for production of aroma in butter. Some of these are: Essence

of hazelnuts, essence of bitter almonds (benzoic aldehyde), essence of mirbane (nitro-benzene), coumarin, vanillin, butyric acid and its esters (monobutyrim, *iso*-propyl butyrate, etc.). He noted that none of the above products, either alone or in combination, duplicates the exact odor of butter and suggested that the best technic for developing the aroma of butter in a fatty material is to initiate a natural fermentation which permits development of the aromatic substances *in situ*.

Since lactic acid is odorless it cannot influence the aroma of butter. However, when present in appreciable amounts, it definitely affects the taste.

#### FACTORS INFLUENCING ACETYLMETHYLCARBINOL AND DIACETYL CONTENTS OF BUTTER

A series of factors related to the manufacturing procedure have an influence on the amc and ac<sub>2</sub> contents of butter and thus, presumably, on the flavor of the butter.

##### *Influence of Strain of Butter Culture*

Practical experience indicates that there are wide variations in the butter cultures used in different plants. In some cases cultures have definite defects, lack of flavor being the most common (43; p. 142), but even with normal cultures there are flavor variations (43; p. 102) of significance. These variations may not be interpreted the same by different persons, one culture in a series being preferred by one person and another by some one else. Because of variations in a culture from day to day, due to the acidities developed and other factors, the cultures in a series may not be rated the same on different days. In general, an experienced person can very accurately judge the value of a particular culture for development of flavor in butter.

Walts (131) compared nine commercial cultures and found there was no particular culture which was superior to the others as far as flavor of the butter was concerned. Knudsen (57) noted a close correlation between the aroma of butter and the aroma of the culture used.

Slatter and Hammer (117) reported an increase in the amc + ac<sub>2</sub> contents of salted and unsalted butter with an increase in the amount of culture employed; this suggested that the flavoring materials in the butter come largely from the culture rather than from changes in the cream during holding.

Krenn (58) added 5 per cent of three cultures containing 0.595, 0.71 and 3.98 mg. ac<sub>2</sub> per l., respectively, to three lots of cream. The cream was ripened and before churning contained 1.85, 1.012 and 2.42 mg. ac<sub>2</sub> per l., respectively. The amounts of ac<sub>2</sub> contributed by the cultures were 0.02975, 0.0357 and 0.199 mg., respectively, while the amounts formed in the cream were 1.82, 0.9763 and 2.22 mg., respectively.

Results obtained by Prill and Hammer (98) showed that, when culture was added to sweet cream, the cream-culture mixture contained approximately the amount of amc contributed by the cream and the culture, whereas the  $ac_2$  content was regularly higher than the calculated amount. The authors suggested that incorporation of air during the mixing presumably made it possible for the organisms to effect considerable oxidation. After holding overnight at  $36^\circ$  to  $44^\circ$  F., the  $ac_2$  had increased further, often more than doubling, and the amc also showed some increase but the percentage increase was not as great as with  $ac_2$ . No close relationship was noted between the  $ac_2$  contents of the cultures and of the finished butter with churnings made by adding the cultures to sweet cream. Results obtained with culture and sour cream showed that more  $ac_2$  was contributed to the cream-culture mixture by the neutralized and pasteurized cream than by the culture; amc was contributed in comparable amounts from the two sources. However, the culture supplied active organisms which made possible a further accumulation of the two compounds. Changes occurring in the  $ac_2$  and amc contents were similar to those obtained with mixtures of sweet cream and culture.

In the trials of Hedrick and Hammer (45) on the ripening of sweet and sour cream for unsalted butter, the amc and  $ac_2$  contents developed by different cultures varied rather widely in some instances and were rather uniform in others. When the same cultures were used in several trials they did not always rank the same on the basis of production of amc or  $ac_2$ , and the variations were not explainable on the basis of the slight differences in acidities of the ripened cream. Also, with the same cultures being used in several trials, each of them sometimes showed relatively high values and sometimes relatively low values.

#### *Influence of Acidity of Cream*

Investigations have clearly established that in a butter culture relatively large amounts of amc and  $ac_2$  are only produced when considerable acid has developed (43; p. 114). This general relationship also applies in the ripening of cream. Because of it also, the amc and  $ac_2$  contents of the original cream are significant only when the cream has developed some acid.

Tapernaux (120) stated that during the course of cream ripening acid develops and there appears a particular aroma due to the lactic acid organisms. He detected  $ac_2$  in ripened cream; it was most evident when the ripening was not preceded by pasteurization. According to Kay (54) acid formation in cream is essential for aroma production since sweet cream does not contain amc. In the experiments of Barnicoat (6) butter made from cream ripened with culture showed amc +  $ac_2$  contents which paralleled the rise in acid during the ripening.

Michaelian and Hammer (73) found the amounts of amc +  $ac_2$  in sour cream delivered to butter plants was relatively high. They stated that this

was to be expected because of the wide distribution of flavor organisms and the favorable conditions provided by the acid cream for production of  $\text{amc} + \text{ac}_2$ .

Barnicoat (6) indicated that there is little  $\text{amc} + \text{ac}_2$  developed when cream is ripened to the small extent common in the manufacture of mild-flavored butter. Most of the flavor-producing substances present in butter made from slightly ripened cream are not developed during the ripening but are added with the culture. This was demonstrated by experiments in which butter made from cream containing only 0.5 per cent culture, and in which no acid was developed, had a distinct aroma.

In the trials of Bouska (10) cream which had soured spontaneously for a moderate period, and which was churned without culture, produced butter with aroma. However, cream which had soured so long that  $\text{amc}$  and  $\text{ac}_2$  had disappeared did not produce aromatic butter unless culture was added. Bungler (16) and also Mohr and Wellm (79) noted that butter made from sweet cream had much lower  $\text{amc}$  and  $\text{ac}_2$  contents than butter made from sour cream. Makar'in (67) reported that the  $\text{amc}$  and  $\text{ac}_2$  contents increased sharply during souring of cream; the same was true during souring of skimmilk.

Davies (24) stated that the rate of formation of  $\text{ac}_2$  in cream can best be measured by calculating the ratio of  $\text{ac}_2$  to acid ( $\text{ac}_2$  as ppm. and acid as per cent lactic). When cream was ripened for 3 hours at  $89.6^\circ \text{F}$ . with a cheese culture the ratio was 3, with a butter culture it was 6 and with a *S. cremoris* culture it was 24; acidities were 0.25, 0.24 and 0.20 per cent, respectively. Incubating for longer periods showed that the  $\text{ac}_2$  content of the *S. cremoris* culture increased more rapidly than that of the other two cultures with less increase in acid. The total  $\text{ac}_2$  and  $\text{amc}$  in each culture increased roughly with acid, but at different rates. In 6 hours the  $\text{ac}_2$  to  $\text{amc}$  ratio was 1:40 with the cheese culture, 1:20 with the butter culture and 1:18 with the *S. cremoris* culture. Davies (25) also noted that the most desirable property of a culture is that of forming the largest amount of  $\text{ac}_2$  at a low acidity since in higher acid ranges it is the  $\text{amc}$  which is formed in largest amount; in general, at low acidities the ratio of  $\text{ac}_2$  to  $\text{amc}$  is greater than at higher acidities.

According to Davies (25) when a culture is used to acidify cream, the titrable acidities of the cream which correspond to flavorless butter, mild-flavored butter, medium-flavored butter and full-flavored butter are 0.10 to 0.15, 0.15 to 0.25, 0.25 to 0.40 and 0.40 to 0.75 per cent, respectively. These figures apply to cream containing about 35 per cent fat. The cream serum acidities would be 0.13 to 0.20, 0.20 to 0.30, 0.30 to 0.50 and above 0.50 per cent, respectively. Various factors other than acidity which influence the degree of flavor development were listed as: (a) Distribution of the various types of lactic acid bacteria in the culture; (b) their virility under com-



petitive conditions in cream; (c) citric acid and oxygen contents of the cream; and (d) degree of biochemical and oxidation reactions occurring in the ensuing butter. Later, Davies (27) reported that the following titrable acidities in cream will ensure the various degrees of butter flavor: Mild flavor, 0.20 to 0.25 per cent; medium flavor, 0.25 to 0.35 per cent; and full flavor, 0.35 to 0.55 per cent. The corresponding  $ac_2$  contents of lots of butter of ascending degrees of flavor were: Mild flavor, 0.5 to 1.5 ppm.; medium flavor, 1.5 to 2.5 ppm.; and full flavor, 2.5 to 4.0 ppm. These conditions referred to cream of 30 to 38 per cent fat, pasteurized by the flash method at 180° to 190° F., cooled and ripened at 65° to 70° F. to the desired acidity with butter culture. The author stated that if depth of flavor of butter is of importance, it is necessary to produce as much  $ac_2$  as possible during the initial stages of cream ripening. That is, the flavor organisms must produce aroma before *S. lactis*, which produces acid only, depletes the cream of its dissolved oxygen. The ratio of  $ac_2$  to amc was believed to depend on oxidation-reduction reactions occurring in the cream and these are governed, to an appreciable extent, by the oxygen tension in the cream.

Results of laboratory trials by Hedrick and Hammer (45) on sweet cream ripened to various acidities at 50° to 70° F. showed that as the acidity of the cream increased the  $ac_2$  content increased in 29 (81%) of the 36 trials and the amc content in 32 trials (89%). Most of the increases were significant, but some of them were very small. Both  $ac_2$  and amc increases were small in the pH range 6.5 to 5.5. In the remaining trials the  $ac_2$  and amc contents showed changes other than regular increases as the acidity of the cream increased.  $Ac_2$  contents of six lots of sweet cream with added culture held for a normal ripening period at 32° F. ranged from 0.12 to 0.28 ppm. (average 0.22 ppm.); the amc contents varied from 3.5 to 16.5 ppm. (average 10.6 ppm.). Three trials were carried out with neutralized sour cream ripened to various acidities at 58° to 62° F. Three acidities were used in each of two trials and four acidities were employed in one trial; in each trial a portion of the cream-culture mixture was held at 32° F. for the normal ripening period. An increase in acidity resulted in an increase in  $ac_2$  and also in amc in each case. Increases in  $ac_2$  were conspicuous and in two of the trials increases in amc were large. The  $ac_2$  and amc contents of the three lots of neutralized sour cream with added culture held at 32° F. generally were higher than those of sweet cream with added culture, the average  $ac_2$  content being 0.42 ppm. (from 0.35 to 0.55 ppm.) and the average amc content 22.1 ppm. (from 20.9 to 23.3 ppm). This relationship is explained by development of the two compounds during the souring of the cream.

In ten trials on a semi-commercial scale Hedrick and Hammer (45) ripened sweet cream (seven trials) and sour neutralized cream (three trials) to various acidities at 50° to 55° F. Three acidities were employed in three of the trials with sweet cream and two acidities were used in the

remaining trials. In two trials with sour cream,  $ac_2$  and amc determinations were made just after pasteurization and again after addition of culture. With an increase in acid there was an increase in  $ac_2$  in nine trials (90%) and in amc in seven trials (70%). Some of the increases were small.  $Ac_2$  decreased in one trial (10%), the drop being from 1.88 to 1.49 ppm. with an increase in acid from 0.37 to 0.49 per cent. Amc decreased in three trials (30%), in one instance after a striking increase; two of the trials involved sour cream in which the citric acid may have been completely fermented. In general, as the  $ac_2$  increased during the ripening of cream the amc also increased, but in some instances there was an increase in  $ac_2$  and a decrease in amc. Commonly, increases in  $ac_2$  and amc were in the same general proportion, but again there were exceptions.

The effect of ripening acidity on the score of unsalted butter was studied by Hedrick and Hammer (45). When the butter was held 1 week at 36° to 40° F., ripening cream to the higher of the two acidities used in each trial gave butter which scored 0.25 to 0.75 of a point higher than ripening to the lower acidity in five (71%) of the seven trials; there was no difference in score in one trial (14%) in which the acidities of the two lots of cream differed only 0.04 per cent; and in one trial (14%) the butter from the higher acid cream scored 0.25 of a point lower but the difference in the cream acidities was small, although both were relatively high (0.48 and 0.51%). After 1 month at 36° to 40° F., the butter made from the higher acid cream scored 0.25 to 0.5 of a point higher in three trials (43%), no difference in score was noted in two trials (29%) and the butter from the higher acid cream scored 0.5 of a point lower in two trials (29%). After 6 months at -10° to 0° F., ripening to the higher acidity gave butter which scored from 0.25 to 0.75 of a point higher in all trials.

#### *Influence of Temperature of Ripening*

Various investigations have dealt with the effect of ripening temperature on production of amc and  $ac_2$  in butter cultures and similar studies have been carried out in connection with cream ripening.

Bunger (16) reported that cream allowed to sour at 62.6° F. had the highest content of amc and  $ac_2$ .

Wiley (135) inoculated sweet cream with 10 per cent culture and incubated it at 80° F. When ripened to pH 5, the cream contained about 0.8 ppm.  $ac_2$  and 18 ppm. amc. Pasteurized sweet cream ripened with culture to pH 5 and then held 24 hours at 48° F. contained about 4.0 ppm.  $ac_2$  and 90 ppm. amc.

Mohr and Wellm (79) noted that the  $ac_2$  content of butter varied with the ripening temperature of the cream. Temperatures of 62.6° F., 50° F. and a combination of 62.6° and 50° F. were studied. Butter made from cream ripened at 62.6° F. contained 0.36 mg.  $ac_2$  per kg. and 2.76 mg.

$\text{amc} + \text{ac}_2$ ; these were the largest amounts found. According to Mazé (71) the most favorable temperature for development of flavor and aroma substances in cream is  $57.2^\circ$  to  $60.8^\circ$  F.

Fabricius and Hammer (33) determined the amounts of  $\text{amc} + \text{ac}_2$  in cream held overnight at  $28^\circ$  to  $36^\circ$  F. after adding 8 per cent culture, in cream held overnight at  $42^\circ$  to  $51^\circ$  F. after adding 8 per cent culture, and in cream ripened 1 hour at  $70^\circ$  F. and then cooled and held at  $28^\circ$  to  $36^\circ$  F. after adding 8 per cent culture. Results showed that the unripened cream held at  $42^\circ$  to  $51^\circ$  F. usually was higher in  $\text{amc} + \text{ac}_2$  than the unripened cream held at  $28^\circ$  to  $36^\circ$  F. and contained about the same amounts as the ripened cream held at  $28^\circ$  to  $36^\circ$  F. Amounts of  $\text{amc} + \text{ac}_2$  in cream held cold after adding culture 16 hours before churning and in cream to which culture was added at churning also were compared. The higher  $\text{amc} + \text{ac}_2$  contents were found with addition of culture 16 hours before churning. Another series of comparisons involved cream held at a low temperature for 3, 6, or 16 hours after adding culture; there were both increases and decreases in  $\text{amc} + \text{ac}_2$  contents as a result of the holding. Effects of holding for 64, 70, 88 or 112 hours also were variable. Immediately after adding culture the  $\text{amc} + \text{ac}_2$  contents of the different portions in a trial were essentially the same.

Laboratory tests on the effect of ripening temperature on the  $\text{ac}_2$  and  $\text{amc}$  contents of cream were made by Hedrick and Hammer (45). In 18 trials sweet cream was ripened at  $50^\circ$ ,  $60^\circ$  and  $70^\circ$  F. Ripening periods varied from 5 to 45 hours, depending on the temperature and the degree of acid desired.  $\text{Ac}_2$  and  $\text{amc}$  determinations were made soon after the desired acidity was reached. The highest  $\text{ac}_2$  content developed at  $50^\circ$  F. and the lowest at  $70^\circ$  F. in 14 of the trials (78%); the same relationship occurred with  $\text{amc}$  in 11 trials (61%). Both  $\text{ac}_2$  and  $\text{amc}$  production were highest at  $50^\circ$  F. and lowest at  $60^\circ$  F. in two trials (11%). The highest  $\text{ac}_2$  content was produced at  $60^\circ$  F. and the lowest at  $70^\circ$  F. in one trial (6%); the same relationship occurred with  $\text{amc}$  in five trials (28%).  $\text{Ac}_2$  production was highest at  $70^\circ$  F. and lowest at  $60^\circ$  F. in one trial (6%). Variations in  $\text{amc}$  production at the three temperatures were small and definitely less on a percentage basis than variations in  $\text{ac}_2$  production. Four trials were carried out on a semi-commercial scale; three involved sweet cream and one involved neutralized sour cream.  $\text{Ac}_2$  production in cream ripened at  $62^\circ$  F. was larger than in cream ripened at  $70^\circ$  F. in two trials (50%) and smaller in two trials (50%).  $\text{Amc}$  production showed similar variations. Differences in production of  $\text{ac}_2$  and  $\text{amc}$  in a comparison commonly were small. In three trials, 0.1 per cent citric acid was added to the cream and the cream agitated during ripening overnight to an acidity of 0.45 per cent.  $\text{Ac}_2$  and  $\text{amc}$  production was much greater in each trial than in the cream ripened at either  $62^\circ$  or  $70^\circ$  F.

Effect of ripening temperature on the score of the resulting unsalted butter was studied by Hedrick and Hammer (45). With butter held 1 week at 36° to 40° F., ripening of the cream at 62° F., rather than 70° F., gave a score increase of 0.25 to 0.75 of a point in two trials (50%), no difference in score in one trial (25%) and a score decrease of 1 point in one trial (25%). With butter held 1 month at 36° to 40° F., ripening at 62° F. gave a score increase of 0.5 to 0.75 of a point in each of three trials (100%). With butter held 6 months at -10° to 0° F., ripening at 62° F. gave a score increase of 0.5 of a point in each of two trials (50%) and a score decrease of 0.75 and 1.0 point in two trials (50%).

Hoecker and Hammer (47) found that on holding cream plus culture 16 hours at 40° F., the  $ac_2$  and amc contents increased when butter culture, *S. diacetylactis* or *S. citrophilus* was used; decreases often occurred with *S. citrovorus* or *S. paracitrovorus*; usually little or no change occurred with an unidentified organism or *S. aromaticus*.

#### *Influence of Fat Content of Cream*

Variations in the fat contents of different lots of cream are large enough to be of importance from the standpoint of cream ripening and butter manufacture. With a high fat content the serum content is small, and a certain percentage of butter culture, based on the cream, has a greater effect in lowering the pH, etc., than when the fat content is low and the serum content correspondingly high. On the other hand, with a high serum content the percentage of citric acid in the cream is larger.

Orla-Jensen (85) stated that since the lactic acid bacteria do not act on butterfat they must produce the characteristic butter aroma and acid from the other constituents of the milk; therefore, the richer the cream, the less aroma will be formed therein.

Barnicoat (7) investigated the effect of the growth medium on development of  $ac_2$  and amc. Experiments made with milk and cream steamed for 30 to 40 minutes, allowed to cool and inoculated with 0.5 per cent culture showed that there was no essential difference, with due allowance for the fat content of the cream, in the action of culture in skimmilk, whole milk and cream.

$Ac_2$  and amc contents of butter churned from whole milk and from cream of varying fat contents were determined by Mohr and Wellm (79). Butter made from 40 per cent cream contained the most  $ac_2$ , while butter made from 20 per cent cream and that made from whole milk were about equal in  $ac_2$  contents. Amc contents of the lots of butter increased with increasing fat contents of the cream. Bunger (16) noted that on churning sour whole milk, 20 per cent cream and 40 per cent cream, the  $ac_2$  content was highest in the butter from the 40 per cent cream; butter made from the whole milk and that made from 20 per cent cream contained practically the same amounts of  $ac_2$ .

Bogdanow (9) recommended an increase in the fat content of the cream in order to obtain increased aroma. He stated that aroma is absorbed by the fat.

*Influence of Addition of Citric Acid to Cream*

Since citric acid is the important source of flavor materials in butter cultures (43; p. 116) and the amount in milk is comparatively small (43; p. 102), this acid or its sodium salt sometimes is added to milk intended for culture. Such an addition also would be expected to have an effect in the ripening of cream.

Preliminary experiments from the Union of South Africa (3) indicated that cream churned with added citric acid gives butter of better flavor than that from untreated cream. Orla-Jensen (85) reported that autolysed yeast extract added to cream did not afford a sure means of increasing the aroma of butter but addition of citric acid gave more promising results. Templeton (121) found that addition of citric acid or sodium citrate to butter culture, to the cream used for butter or to both gave butter with a more pronounced flavor and aroma. Addition of 0.2 per cent citric acid, or its equivalent of sodium citrate, to the culture alone had a slight effect. The effect was more pronounced when the same proportion of citric acid or citrate was added to the cream.

Khubchandani (55) noted that addition of citric acid or sodium citrate to cream resulted in butter with increased aroma but poorer keeping qualities. Hunziker (48; p. 364) concluded that, in the absence of more favorable experimental data, addition of citric acid to cream appears to be of doubtful merit; he suggested avoiding the expense until more convincing knowledge of the merits of the addition is available.

Experiments involving addition of citric acid to cream at the time of inoculating with culture were reported by Hedrick and Hammer (45). In 14 laboratory trials sweet cream was ripened with and without added citric acid to about the same acidity at temperatures from 54° to 60° F.; 0.05 per cent acid was added except in one trial in which 0.10 per cent was used. Addition of citric acid gave a higher  $ac_2$  content in the cream in all the trials (100%) and a higher amc content in 12 trials (86%). With both  $ac_2$  and amc some of the increases were very small. In eight trials neutralized sour cream was ripened with and without citric acid to about the same acidity at temperatures from 58° to 63° F. In six trials (75%) added citric acid resulted in an increase in the  $ac_2$  content. In the four trials involving three amounts of acid, the increases were roughly in proportion to the amount added; in one trial (12.5%) 0.05 and 0.10 per cent added citric acid did not increase the  $ac_2$  content but 0.15 per cent did, and in one trial (12.5%) 0.10 and 0.15 per cent citric acid gave the same  $ac_2$  content. Added citric acid regularly increased the amc content and, in the trials in which three amounts were used, the increases were roughly in proportion to the amount

added. Data were obtained on seven semi-commercial trials in which cream was ripened with and without added citric acid (0.05%) to about the same acidity at temperatures from 50° to 54° F. Added citric acid increased the  $ac_2$  content in six comparisons (86%); the increases varied widely, with three of them being 0.20 ppm. or less. The one decrease in  $ac_2$  content was very small, being 0.13 ppm. Added citric acid increased the amc content in three comparisons (43%), the increases ranging from 12.8 to 34.8 ppm. In one trial (14%) the amc content was the same with and without added citric acid. In the three comparisons (43%) in which addition of the acid resulted in a decrease in amc content, the decreases ranged from 4.0 to 15.8 ppm.

Hedrick and Hammer (45) studied the effect of adding 0.05 to 0.10 per cent citric acid to cream on the score of the resulting unsalted butter. With butter held 1 week at 36° to 40° F., addition of citric acid increased the score 0.25 to 0.50 of a point in four trials (57%) and there was no difference in score in three trials (43%). With holding 1 month at 36° to 40° F. the addition gave a score improvement of 0.25 to 0.75 of a point in six trials (86%) and no difference in score in one trial (14%). With holding 6 months at -10° to 0° F. the addition resulted in an increase in score of 0.5 to 0.75 of a point in six trials (86%) and a score decrease of 0.5 of a point in one trial (14%).

#### *Influence of Aeration of Cream*

The oxygen supply is a factor in the production of amc and  $ac_2$  in butter cultures (43; p. 118) and, accordingly, should be important in flavor development in cream. However, aeration may have an undesirable influence on the flavor of some of the milk constituents because of their tendency to oxidize.

According to Orla-Jensen (85) aeration of cream may possibly promote production of aroma; it increased the volatile acidity of cream. Davies (23) noted that most of the  $ac_2$  in cream is formed while there is considerable atmospheric oxygen in solution.

Virtanen and Tarnanen (129) considered that the fact  $ac_2$  is formed only when oxygen acts as the hydrogen acceptor should be considered in butter manufacture. They suggested that cream be ripened under as aerobic conditions as possible and that churning be carried out so as to ensure a maximum content of air in the butter. (See also 128.) Virtanen (128) found that passing sterile air into cream during ripening had a very favorable effect on the aroma of fresh butter, the  $ac_2$  content being increased considerably. Makar'in (67) stated that the upper layers of souring cream are richer in  $ac_2$  and amc than the lower layers.

Davies (24) reported that formation of amc was favored by a low oxygen content. Later, he (25) indicated that when a culture was added to cream,

the important factor was the rate at which the aroma-producing organisms proliferated before the true lactic acid bacteria induced a low oxygen content.  $\text{Ac}_2$  was found in cream in greatest amounts when there still was some free oxygen present.

It was noted by Prill and Hammer (98) that when sweet cream and aerated-pressure (p. 89) or modified aerated-pressure (p. 88) culture were mixed, analyses did not indicate an increase in  $\text{ac}_2$  during the mixing but sometimes suggested a slight decrease, especially in the case of aerated-pressure culture. During the holding of the cream-culture mixtures overnight and during churning, the organisms usually produced additional  $\text{ac}_2$  and  $\text{amc}$ ; although in most cases the actual increment in  $\text{ac}_2$  was as great (or greater) than when regular butter culture was used, the percentage increase was not because of the relatively large amount of  $\text{ac}_2$  originally present. It appeared that if the cream had been churned soon after addition of culture, aerated-pressure or modified aerated-pressure culture probably would show a greater practical advantage over regular butter culture.

Laboratory trials on the effect of agitation during the ripening of cream on the  $\text{ac}_2$  and  $\text{amc}$  contents were reported by Hedrick and Hammer (45). In nine trials (seven with sweet cream and two with neutralized sour cream) cream was ripened with and without agitation at 60°, 63° or 70° F. At 60° or 63° F. the cream was agitated three times during the ripening while at 70° F. it was agitated every hour for 3 or 4 hours. In each trial the  $\text{ac}_2$  and  $\text{amc}$  contents of the agitated cream were much higher than those of the unagitated control. Essentially the same acidity developed with and without agitation. Effect of agitation with added citric acid was studied in six trials (four with sweet cream and two with neutralized sour cream); 0.10 per cent citric acid was used and the ripening temperature was 60°, 63° or 70° F. As without addition of citric acid, agitation greatly increased both the  $\text{ac}_2$  and  $\text{amc}$  contents but had no appreciable effect on acid development. Five trials were carried out on a semi-commercial scale (three with sweet cream and two with neutralized sour cream). Again the  $\text{ac}_2$  and  $\text{amc}$  contents were greatly increased by the agitation whereas the acid development was not significantly influenced.

In a study of the effect of agitation during ripening on the score of the resulting unsalted butter after 1 week at 36° to 40° F., Hedrick and Hammer (45) found that agitation of the cream gave a score increase of 0.25 to 0.75 of a point in three (60%) of the five trials and no difference in score in two trials (40%). With holding of the butter 1 month at 36° to 40° F., agitation gave a score increase of 0.5 to 0.75 of a point in two trials (40%), no difference in score in two trials (40%) and a score decrease of 0.25 of a point in one trial (20%); with holding 6 months at -10° to 0° F., agitation resulted in a score increase in four trials (80%) and no difference in one trial (20%).

Hoecker and Hammer (47) reported that immediately after mixing the  $ac_2$  contents of cream with added butter culture were sometimes higher and sometimes lower than the theoretical amounts calculated from the  $ac_2$  contents of the cream and culture; in most trials the amc contents were about the same as the theoretical values but in some instances were higher.

#### *Influence of Neutralization and Pasteurization of Cream*

Neutralization and pasteurization of cream constitute the primary treatments of much of the cream intended for butter, neutralization now being employed even with some of the sweet cream used for butter. In the investigations of the effect of these processes on the amc and  $ac_2$  contents of cream, there has been some variation in the results, probably because of variations in the types of cream used and in the neutralization and pasteurization procedures.

Barnicoat (7) found that neutralization and pasteurization of cream caused no notable decrease in  $ac_2$  but a distinct decrease in amc. Destruction of amc was correlated with the neutralization point of the cream and was similar with the two types of pasteurizers used; it was thought to have some bearing on the opinion, frequently expressed, that butter made from over-neutralized cream often is deficient in flavor.

Davies (24) noted that cream which had developed certain amounts of amc and  $ac_2$  did not yield butter having flavor or aroma when the cream was neutralized to 0.10 to 0.15 per cent acid and flash pasteurized. Disappearance of amc and  $ac_2$  from the cream was explained on the basis of volatility of the aroma constituents and absence of oxidation of amc in the butter. Examination of many samples of neutralized cream butter after 6 months in storage revealed no trace of  $ac_2$  or amc +  $ac_2$  by the usual analytical methods. Later Davies (26) stated that butter made from neutralized cream possesses no butter flavor; if flavor is required, the pasteurized and cooled cream is ripened by addition of culture to different acidities, depending on the degree of flavor desired.

According to Prill and Hammer (98) neutralized and pasteurized cream frequently had a lower  $ac_2$  content than the raw cream. With cream having an original acidity of 0.5 to 0.6 per cent, it was found that after preliminary neutralization to 0.25 per cent acid, or during the first part of the heating, the  $ac_2$  content fell to a very low value and the amc content commonly decreased slightly. The compounds may have been reduced to 2,3-butylene glycol. When the cream had been heated to pasteurizing temperature, and also in subsequent examinations, the  $ac_2$  contents showed very significant increases. The amc content showed an increase when the cream had reached pasteurizing temperature and then remained fairly constant in the subsequent examinations. The authors stated that the increase in  $ac_2$  may possibly have been caused by non-biological oxidation but the increase in



ame through non-biological reactions seemed improbable. Net result of the cream processing was a decrease in  $ac_2$  content and relatively little change in ame content.

#### *Influence of Certain Metallic Salts*

Mohr and Arbes (77) noted that when milk or cream was treated with certain metallic salts and then soured and stored for approximately 20 hours, or allowed to sour during such storage, the  $ac_2$  content was increased over that of the control lot. The salts tested, in order of increasing effectiveness, were cupric chloride, manganese chloride, ferric chloride and a combination of all three; they were added in amounts of 0.5 to 2.0 mg. per l. of milk or cream. Addition of the salts to milk or cream immediately before determining the  $ac_2$  was without effect on the results.

#### *Influence of Churning*

Because of the agitation of the cream and the butter granules in the presence of air, churning would be expected to have an effect on the  $ac_2$  contents of the cream and the resulting butter and perhaps on the ame contents.

Tapernaux (120) stated that the butter formed by agglomeration of fat globules from the cream during churning seems to retain the aromatic substances, and the aroma of butter continues to increase in intensity throughout the days which follow manufacture; the butter also contains fermentable substances and ferments. According to Davies (22)  $ac_2$  probably is not formed until butter is churned and stored. Bunger (16) noted that the  $ac_2$  content of cream increased during churning.

Mazé (71) considered that churning must be regarded as an additional aerobic fermentation, its object being to oxidize the reductive products of the anaerobic lactic acid fermentation and to produce the flavor characteristic of fresh, first-quality butter. Results obtained by Mohr and Wellm (79) indicated that the largest contents of  $ac_2$  and ame were obtained in fresh butter at a churning temperature of 57.2° F.

Virtanen (128) stated that churning of cream in a churn filled with some indifferent gas is obviously not suited to production of butter with a rich aroma. He suggested that churning should be carried out in such manner as to ensure a maximum content of air in the butter since this promotes formation of ame and finally  $ac_2$ .

The analyses of Prill and Hammer (98) showed an increase in  $ac_2$  content during churning. The authors noted that the ame content would not be expected to change greatly in the short period involved.

#### *Influence of Washing Butter with Water*

Because of the solubility of ame and  $ac_2$  in water, the washing of butter would be expected to remove some of these materials from it.

In 1893, Conn (20) noted that the flavor of butter was very much more prominent without thorough washing than with it. If the butter was washed long enough, all of the aroma could be washed away.

Leitch (60) indicated that much of the aroma of butter is lost during the washing. He recommended washing butter with serum prepared by fermenting pasteurized skimmilk with culture, removing the curd and filtering the liquid through sterile linen. Testoni and Ciusa (123) found that when butter was washed four times it no longer contained  $ac_2$ ; when washed only twice it contained the compound.

According to results obtained by Barnicoat (6) no extraction of  $amc + ac_2$  appeared to take place during the washing of butter. Later he (7) reported the loss of  $amc + ac_2$  by the washing of butter was relatively unimportant.

Kreim (58) noted that butter wash water contained considerable  $ac_2$ . Bungler (16) removed the greater part of the  $amc$  and  $ac_2$  from butter granules by washing them three times. Makar'in (67) reported that washing butter greatly reduced the  $ac_2$  content. In the analyses of Mohr and Wellm (79) unwashed butter contained 1.69 mg.  $ac_2$  per kg. and 9.30 mg.  $amc + ac_2$  whereas washed butter contained 0.86 mg.  $ac_2$  and 3.78 mg.  $amc + ac_2$ . According to Pont (94) approximately four-fifths of the  $amc + ac_2$  originally present is removed by the buttermilk and the washing of the butter.

Mohr and Arbes (77) washed butter with water containing 0.5 to 1.5 mg. per l. of various salts of iron, manganese and copper; they concluded that the salts had no effect on the  $ac_2$  content of the butter.

Prill and Hammer (98) analyzed several samples of butter wash water and found only a fraction of a mg. of  $amc + ac_2$  per kg. They stated that while the total  $amc + ac_2$  in wash water may be significant, compared to the amount present in the butter, it is difficult to differentiate between material which comes from the film of buttermilk remaining between the butter granules and that which might possibly be removed from the granules themselves. If  $amc$  and  $ac_2$  are both held entirely in the serum droplets in the same manner, they should be retained in the butter in the same proportion as they exist in the churning mixture at the time the butter is formed, and any possible washing out of the compounds should also be in this proportion.

#### *Partition of Acetylmethylcarbinol and Diacetyl During Churning*

When cream is churned, some of the  $amc$  and  $ac_2$  goes into the butter and some into the buttermilk. This general relationship has led to various studies on the partition of the compounds during churning. Presumably, from the standpoint of butter flavor, it would be better if all the  $amc$  and  $ac_2$  present in the cream were retained in the butter.

**Comparative amounts of acetylmethylcarbinol and diacetyl in cream and butter.** Barnicoat (6) calculated that about one-fifth of the  $amc + ac_2$

in cream is carried into the butter. Michaelian and Hammer (73) noted that butter regularly contained much less  $\text{amc} + \text{ac}_2$  than the cream from which it was churned. With four lots of butter the  $\text{amc} + \text{ac}_2$  was not measurable with the gravimetric method then commonly in use but with the other 52 lots in the series the ratios of  $\text{amc} - \text{ac}_2$  contents of the cream to those of the butter ranged from 1:0.032 to 1:0.218. The ratios were not correlated with the amount of  $\text{amc} - \text{ac}_2$  in the cream or the acidity of the cream.

Results obtained by Mohler and Herzfeld (76) indicated that cream from which the butter was churned contained about three times more  $\text{ac}_2$  than the resulting butter. Davies (23) reported that only about one-fifth of the  $\text{amc} + \text{ac}_2$  in cream appeared in the butter and about one-fourth of the  $\text{ac}_2$ . Well ripened cream (0.6 per cent acid) containing 5 to 10 ppm.  $\text{ac}_2$  and 100 to 250 ppm.  $\text{amc}$  yielded butter containing 1 to 2 ppm.  $\text{ac}_2$ .

According to Barnicoat (7) the percentage of  $\text{ac}_2$  retained by butter varies from 0 to 33 per cent, the extremes occurring with neutralized creams. Average retention in the butter was 15 per cent of the amount originally in the cream. The ratio of  $\text{ac}_2$  in butter to  $\text{ac}_2$  in cream (30 to 40 per cent fat) was 0.4. The ratio of  $\text{ac}_2$  to  $\text{amc}$  in "mild starter" butter examined 1 day after manufacture was greater than in cream; the average ratio of  $\text{ac}_2$  to  $\text{amc}$  in 11 lots of cream was 0.07 while in the butter the ratio was 0.11.

Brioux and Jouis (14) found that butter contained less  $\text{ac}_2$  than the cream from which it was churned. Bungler (16) noted that from one-fourth to one-sixth of the  $\text{ac}_2$  and from one-fifteenth to one-sixteenth of the  $\text{amc}$  in the cream was present in the resulting butter.

Barnicoat (7) reported that when  $\text{amc}$ , as the synthetic compound, was added to pasteurized cream there was, in the absence of butter culture, a loss before churning. Results obtained on 12 churnings showed that from 7 to 22 per cent (average 14%) of the  $\text{amc}$  in the cream was retained in the butter.

Davies (24, 25, 26) noted that the ratio of  $\text{ac}_2$  in cream to that in butter was approximately 4:1; the ratio of  $\text{amc} + \text{ac}_2$  in cream to that in butter was between 4:1 and 5:1.

Analyses made by Mohr and Wellm (79) on washed and worked butter showed that the butter had only one-fourth to one-sixth of the  $\text{ac}_2$  content of the cream and from one-fifteenth to one-sixteenth of the  $\text{amc}$  content.

Krenn (58) determined the amounts of  $\text{ac}_2$  in butter obtained from cream which contained 1.012 and 2.42 mg.  $\text{ac}_2$  per l., respectively. The butter contained 0.357 and 0.00 mg.  $\text{ac}_2$  per kg., respectively. Krenn concluded that only a small part of the  $\text{ac}_2$  formed during the ripening of cream is present in the resulting butter. Brioux and Jouis (14) found that cream containing 95.2 mg.  $\text{amc}$  per kg. yielded butter containing 42.4 mg.; the same cream contained 1.92 mg.  $\text{ac}_2$  and the resulting butter 1.50 mg.  $\text{ac}_2$ .

According to Davies (25) the amount of  $ac_2$  in butter is roughly 10 per cent of that in the cream. The amount in the finished butter was considered to be influenced by the amount of washing and the fraction of the moisture accounted for by the buttermilk droplets which, in turn, depends on the degree of working. The author further stated (27) that the distribution of  $ac_2$  in butter is roughly proportional to the ratio of butter serum to buttermilk.

Results of Hoecker and Hammer (46) showed that only small percentages of the  $amc$  and  $ac_2$  in a cream-culture mixture were retained in the butter, the remainder being in the buttermilk; the percentage retention was essentially the same with various cultures, although with each culture there was considerable variation from one churning to another.

Hedrick and Hammer (45) reported that with 74 semi-commercial churnings of unsalted butter, the minimum, maximum and average ratios of the  $ac_2$  contents of the ripened cream and of the corresponding butter were 1:0.142, 1:0.709 and 1:0.352, respectively; the corresponding ratios for  $amc$  were 1:0.059, 1:0.683 and 1:0.217, respectively. The investigators also noted that the  $ac_2$  and  $amc$  contents of a series of lots of butter sometimes did not follow the same order as the contents of the lots of cream from which the butter was churned. The irregularities were of all possible types. In some cases the  $ac_2$  or  $amc$  content of a lot of butter was lower than that of an earlier lot in the series, although the content of the cream was higher. In other cases a value for  $ac_2$  or  $amc$  was lower than the values on the lots preceding and following it in the series, even when there was no drop in the value for the cream. Still other types of variations occurred. The  $ac_2$  contents of the semi-commercial churnings of butter ranged from 0.16 to 1.46 ppm., while the  $amc$  contents varied from 2.9 to 24.0 ppm. The highest values for both  $ac_2$  and  $amc$  were obtained on a lot of butter made from cream ripened with agitation after addition of 0.1 per cent citric acid.

Hoecker and Hammer (47) stated that, in butter made with butter culture,  $amc$  and  $ac_2$  are derived largely from the culture added to the cream and from fermentation of citric acid during holding or ripening of the cream.

**Comparative amounts of acetylmethylcarbinol and diacetyl in cream and buttermilk.** In 1893, Conn (20) noted that, after churning, nearly all the flavor produced during the ripening of cream was contained in the buttermilk. Butter aroma, therefore, was considered due to changes in some constituent of the cream other than fat.

Hammer (40, 41) stated that, when cream was churned, much of the  $amc$  and  $ac_2$  in it was carried into the buttermilk so that the buttermilk contained more  $amc$  and  $ac_2$  than the cream. Michaelian and Hammer (73) found that fresh buttermilk regularly contained more  $amc + ac_2$  than the cream from

which it was obtained. The ratios of  $\text{amc} + \text{ac}_2$  in the cream to that in the buttermilk ranged from 1:2.0 to 1:3.1. In a series of churnings involving cream of various acidities, the ratios ranged from 1:1.1 to 1:2.1.

According to Davies (23) aeration of cream during churning, and the consequent readjustment of the ratio of  $\text{ac}_2$  to  $\text{amc}$  in the buttermilk, causes the buttermilk to contain more  $\text{ac}_2$  than ripened cream. An increase in the acidity of the buttermilk resulted in a decrease in the  $\text{ac}_2$  content.

Bunger (16) found that buttermilk contained from 2.4 to 4.0 times more  $\text{ac}_2$  than the sour cream before churning. Results obtained by Davies (24) indicated that the ratio of  $\text{ac}_2$  in buttermilk to that in the original cream varies from 2:1 to 3:1, depending on the fat content of the cream and the ratio of  $\text{ac}_2$  to  $\text{amc}$  in the cream.

Krenn (58) determined the amount of  $\text{ac}_2$  in buttermilk obtained from cream which contained 1.012 and 2.42 mg.  $\text{ac}_2$  per l., respectively, before churning. The buttermilk contained 1.250 and 4.098 mg. per l., respectively. Buttermilk having an initial  $\text{ac}_2$  content of 4.59 mg. per l. showed 4.53 mg. after holding 8 hours and 4.65 mg. after holding 24 hours. This increase was believed due to the lactic acid and aroma-forming bacteria which were present in large numbers.

Makar'in (67) noted that the  $\text{ac}_2$  content of buttermilk was greater than that of the cream before churning. Results obtained by Mohr and Wellm (79) showed that buttermilk contained 2.4 to 4.0 times as much  $\text{ac}_2$  as the cream before churning. Brioux and Jouis (14) stated that after churning the  $\text{ac}_2$  and  $\text{amc}$  in fermented cream were contained largely in the buttermilk. Fermented cream containing 95.2 mg.  $\text{amc}$  per kg. yielded buttermilk containing 195.3 mg.; the same cream contained 1.92 mg.  $\text{ac}_2$  and the resulting buttermilk contained 3.10 mg.

**Comparative amounts of acetylmethylcarbinol and diacetyl in butter and buttermilk.** van Niel *et al.* (126) found  $\text{ac}_2$  in a sample of buttermilk having an exceptionally fine aroma and a high  $\text{amc}$  content. According to Hammer (41) there is a concentration of  $\text{amc}$  and  $\text{ac}_2$  in the buttermilk rather than in the butter.

In order to check the accuracy of his investigation, Krenn (58) attempted an  $\text{ac}_2$  balance. In one trial 10 kg. of cream (21% fat) produced 2.42 kg. of butter and 7.58 l. of buttermilk. The cream (9.88 l.) contained 10.0 mg. of  $\text{ac}_2$ , the resulting butter contained 0.86 mg. and the buttermilk (7.58 l.) contained 9.15 mg. Therefore 10.01 mg. of  $\text{ac}_2$  was obtained in all, or 0.01 mg. more than was present in the cream. In another trial 10 kg. of cream (24.5% fat) produced 3.06 kg. of butter and 6.94 l. of buttermilk. The cream (10 l.) contained 24.2 mg. of  $\text{ac}_2$  and the resulting butter contained none; the buttermilk (6.94 l.) contained 28.4 mg. or 4.2 mg. more than the original cream. This result was explained by a conversion of  $\text{amc}$  in the

cream to  $ac_2$  by the carbonic acid present. To clarify this point 2 l. of sour cream containing 4.06 mg. of  $ac_2$  was churned in a hand churn. The buttermilk was drawn completely and the resulting butter was washed three times with water. The buttermilk contained 6.14 mg. of  $ac_2$ , the first wash water 3.16 mg. and the two succeeding wash waters none. The resulting butter contained 0.3 mg.  $ac_2$ . In the trial, 5.54 mg. of  $ac_2$  more than was present in the cream was accounted for. It appeared that  $ac_2$  actually was formed during the churning process by oxidation of amc.

Results obtained by Alberti (1) indicated that considerable amc and  $ac_2$  remained in the buttermilk.

Prill and Hammer (98) noted that certain general relationships applied to the distribution of amc and  $ac_2$  between the butter and the buttermilk in all the churnings studied. The estimated mg.  $ac_2$  in the buttermilk and the butter derived from 1 kg. of churning mixture agreed fairly well with the content of the partly churned mixture and the estimated mg. amc +  $ac_2$  in the two products agreed fairly well with the content just before churning. The  $ac_2$  contents of the butter were very nearly one-fifth those of the corresponding buttermilk. Since butter contains one-fifth serum, it would appear that  $ac_2$  is contained entirely in the serum of butter. However, the amc +  $ac_2$  content of the butter was, in most cases, only about one-tenth that of the buttermilk. The average of the estimated percentages of  $ac_2$  in the churning mixtures that were retained in the butter was 8.8 per cent, and the corresponding value for amc +  $ac_2$  was 4.5 per cent.

**Partition of acetylmethylcarbinol and diacetyl between aqueous and fatty constituents of cream and butter.** Orla-Jensen (85) stated that since the aroma of butter is produced outside the fat globules, it might be expected to wash out as easily as the lactic acid, but this is not the case. Isigny butter, which had a stronger aroma than any other make, was subjected to a very thorough washing during its manufacture. The explanation given was that butterfat can absorb essential oils and other odoriferous substances, both pleasant and unpleasant, and the aroma of the cream therefore passes into the fat globules. It was thought that the greater the proportion of fat globules present, the less aroma would be available for each globule and experience has shown that a high fat percentage is not conducive to production of an aromatic butter.

King (56) found a slow diffusion of  $ac_2$  between the fat and serum phases of butter,  $ac_2$  being dissolved in the fat fraction as well as in the serum. He believed that a slow diffusion of  $ac_2$  from the serum to the fat occurred during storage.

Tapernaux (120) indicated that, during the course of cream fermentation,  $ac_2$  is formed in the water and in the fat and divides itself between the serum and fat so that a part is eliminated in the buttermilk and also in the

wash water. Hammer (40, 41) reported that the amc and  $ac_2$  in butter is mostly in the serum and only very little is in the fat.

According to Barnicoat (6) the final concentration of amc +  $ac_2$  in butter is dependent on the amount of buttermilk retained. He believed that the compounds probably are held in the aqueous portion and are possibly absorbed on the protein rather than dissolved in the fat.

Michaelian and Hammer (73) found the amount of amc +  $ac_2$  in the serum of butter was higher than the amount in the fat. Makar'in (67) stated that  $ac_2$  in butter exists in the butter plasma which was considered to be made up of about one-half buttermilk. In one of eight trials, traces of  $ac_2$  were found in the fat itself.

Davies (24, 25, 27) considered that since the vapor pressure of  $ac_2$  is higher than that of amc a greater amount of  $ac_2$  than of amc would be expected in the fat phase of cream. The amount of  $ac_2$  in the fat, however, was less than the amount in the aqueous phase of butter. The author noted that a considerable amount of both  $ac_2$  and amc occurs in the fat phase.

Mohr and Wellm (79) determined the amounts of amc and  $ac_2$  in butter, butterfat and butter serum which was obtained by careful melting at 113° F. Butter containing 0.56 mg.  $ac_2$  and 14.84 mg. amc +  $ac_2$  per kg. had 0.52 mg.  $ac_2$  and 5.14 mg. amc +  $ac_2$  per kg. in the fat. Butter serum contained 1.24 mg.  $ac_2$  and 61.1 mg. amc +  $ac_2$  per l.

Virtanen (128) stated that  $ac_2$  obviously is dissolved in the water portion of butter and is distributed between the buttermilk and the butter in the same proportions as water.

Results of Barnicoat (7) showed a higher ratio of  $ac_2$  to amc in butter than in the cream from which it was churned. He considered that this observation might indicate a partition of the  $ac_2$  with the fat but the data pointed to an actual development of amc in the butter. Neither  $ac_2$  nor amc was concentrated in the fat during butter manufacture. Makar'in (68) stated that amc and  $ac_2$  are not absorbed by the fat globules but are contained in the aqueous portion of butter. He believed that the aroma of butter was influenced by the concentration of aqueous constituents of the cream retained in the butter; as a rule this represented about 60 per cent of the total moisture content of the butter.

Hoecker and Hammer (47) studied the distribution of  $ac_2$  and amc between "Wesson" oil and water or brine. In the mixtures the water or brine regularly contained higher concentrations of  $ac_2$  and amc than the oil, the differences being greater with amc than with  $ac_2$ . The percentage of  $ac_2$  in a mixture that was contained in the oil was increased by sodium chloride in the water. The concentration of  $ac_2$  in a mixture did not affect the percentage in the oil. Concentrations of amc in the oil were very low and the different concentrations in the mixtures gave essentially the same percentages in the oil. Studies made on the distribution of  $ac_2$  and amc between

butterfat and water or brine showed that the water or brine regularly contained higher concentrations of the compounds than the fat; differences were greater with amc than with  $ac_2$ . As the concentration of  $ac_2$  in a mixture increased, the percentage in the fat did not change appreciably, while as the concentration of amc in a mixture increased, the percentage in the fat decreased. Studies on the distribution of  $ac_2$  and amc between the fat and serum of unsalted and salted butter showed that the serum of butter contained higher concentrations of  $ac_2$  and amc than the fat, with the greater differences again involving the amc. Averages of the percentages of  $ac_2$  and amc contained in the fat were smaller with unsalted than with salted butter. The percentage of  $ac_2$  contained in the fat was independent of the concentration in the butter, whereas the percentage of amc usually decreased somewhat as the concentration in the butter increased. Addition of a solution of  $ac_2$  or a distillate of butter culture to salted butter resulted in essentially the same distribution of  $ac_2$  as when the butter was made from cream containing butter culture. The amounts of  $ac_2$  and amc in butter, as calculated from its composition and analyses of the fat and serum, agreed fairly closely with the determined amounts. In unsalted butter  $ac_2$  and amc concentrations in the serum ranged from 0.25 to 3.55 ppm. and from 4.85 to 119.16 ppm., respectively, and those in the fat varied from 0.19 to 1.11 ppm. and from 0.88 to 6.86 ppm., respectively. The fat contained from 44.4 to 75.0 per cent of the  $ac_2$  and from 26.2 to 46.8 per cent of the amc in the butter. In salted butter  $ac_2$  and amc concentrations in the serum ranged from 0.07 to 2.00 ppm. and from 1.72 to 59.00 ppm., respectively, while those in the fat varied from 0.02 to 0.92 ppm. and from 0.29 to 4.87 ppm., respectively. Of the total  $ac_2$  and amc in the salted butter, the fat contained from 48.5 to 78.4 per cent and from 23.6 to 46.0 per cent, respectively. The investigators concluded that comparative solubilities in the fat probably explain why with  $ac_2$  the concentration in butter does not affect the percentage of the total that is retained in the fat, while with amc an increase in the concentration in butter decreases the percentage of the total that is retained in the fat. The low solubility of amc was believed to limit the amount taken up by the fat. Variations among samples of butter in the percentages of total  $ac_2$  or amc that were contained in the fat were thought to be due to several factors, such as composition of the butter, physical state of the fat, churning procedure and degree to which water is dispersed in the butter; also, analytical errors involved in determining very small quantities of the compounds may be of minor significance. It also was concluded that since large percentages of the  $ac_2$  and amc in cream at churning are removed with the buttermilk, higher concentrations of the compounds would be expected in the serum of butter than in the fat. Although concentrations in the fat were relatively low, the percentages of the compounds in butter that were contained in the fat were comparatively high because butter contains ap-



proximately 80 per cent fat. Partitioning of  $ac_2$  and  $amc$  between the serum and fat was thought to reach an equilibrium in a relatively short time.

### *Influence of Reworking Butter*

Reworking butter has an effect on the action of micro-organisms in it, including the butter culture types (64).

Barnicoat (7) stated that butter which develops off flavors during cold storage usually is considerably improved by reworking. This improvement has been thought to be due to formation of  $ac_2$ ; results of the author, however, showed a slight decrease in  $ac_2$  on reworking butter.

### *Influence of Storage of Butter*

During the holding of butter there are various possibilities in connection with changes in the  $amc$  and  $ac_2$  contents. The compounds may disappear through action of organisms or through chemical reactions. Also, under suitable conditions, they may be formed, particularly through action of the normal butter culture organisms; these organisms are much more active in unsalted than in salted butter (81).

**Salted butter.** Testoni and Ciusa (123) found that the amount of  $ac_2$  in butter decreased on holding and that  $ac_2$  did not develop in butter originally containing none. Tapernaux (120) stated that butter having an intense aroma tends to lose aroma as the holding period is extended and the aroma may even disappear. He suggested that the  $ac_2$  is transformed into  $amc$  by reduction and then into 2,3-butylene glycol. Loss of aroma also was explained by the volatility of  $ac_2$ . Davies (22) reported that  $ac_2$  is lost more rapidly in butter of higher acidity.

According to Barnicoat (6) when butter was made from slightly ripened cream, with or without butter culture, the  $amc + ac_2$  content remained fairly constant throughout the storage (193 days at  $14^\circ$  to  $17^\circ$  F.). Butter made from cream which had been slightly ripened with culture showed some conversion of  $amc$  to  $ac_2$ ; however, in two samples less  $ac_2$  was present at 6 months than at 3 months. All the butter made with culture contained considerable  $amc + ac_2$  (0.7 to 2.3 ppm.). The  $ac_2$  disappearing from butter during storage was considered lost through oxidation to simpler substances. Decomposition of  $ac_2$  was greater in butter made without culture than in butter made with culture. In butter made with culture, a certain amount of  $ac_2$  was lost and considerable of the original  $ac_2$  was reduced to  $amc$ . The author believed that reduction of  $ac_2$  to  $amc$  in butter made with culture was promoted by the reducing action of the culture organisms. The loss of  $ac_2$  in butter made without culture, and to a less extent in butter made with culture, was considered due to oxidation by air. Decomposition of  $ac_2$  in oxidized and tallowy lots of butter was found to be less than might be expected;

this indicated that the fat peroxides may not be as important in the degradation of  $ac_2$  as is generally supposed.

Virtanen and Tarnanen (129) stated that when deterioration in butter is caused by organisms (for example, bacteria of the *Ps. fluorescens* group)  $ac_2$  is lost very quickly; this is evident before other defects become noticeable.

In the studies of Slatter (115)  $amc + ac_2$  usually was not produced in salted butter. No conspicuous increases occurred, even when very little salt was present. Salt appeared to retard the decrease in  $amc + ac_2$  in butter held at various temperatures.

Davies (23) reported that butter made from ripened cream must stand 16 to 24 hours before the maximum aroma develops. This phenomenon occurred at any temperature between 30° and 60° F. Very little increase in aroma occurred at 15° F. The increase in  $ac_2$  after the manufacture of butter was considered to be due to bacterial action on  $amc$  and to autoxidation of  $amc$ . In cream after ripening there is no oxygen in solution but, after churning and working the butter, conditions are different in that the water in butter is saturated with atmospheric oxygen. The organisms in the buttermilk droplets in butter were believed to readjust the  $ac_2$ :  $amc$  ratio to that existing in the early stages of cream ripening so that some of the excess  $amc$  is converted to  $ac_2$ . The high acidity of the buttermilk droplets was thought to cause a slight organic acidity, due largely to oleic acid being split from the fat. Free oleic acid in the presence of atmospheric oxygen was thought to develop small amounts of peroxides which oxidize some of the  $amc$  to  $ac_2$ . After 24 hours, the bacteria of the butter apparently utilize all the oxygen and there is no  $ac_2$  formation. Davies concluded that the degree of flavor is lowered by a gradual oxidation of  $ac_2$  to a flavorless product.

Barnicoat (7) observed no marked change in the  $ac_2$  content of butter during frozen storage for 3 to 4 months. When butter was held at 40° F. for 7 to 10 days after manufacture, the  $ac_2$  content decreased in some cases. Butter in which the greatest increases in  $ac_2$  were observed were made from cream to which  $amc$  had been added and in which the culture was growing fairly vigorously at churning. Development of  $ac_2$  in butter freshly made with culture was considered due to activity of the bacteria or their enzymes. The author concluded that the  $ac_2$  content of butter made with culture, even after cold storage, generally is dependent on the concentration originally present in the cream. When  $ac_2$  was added as the artificial substance, a decrease was observed; butter made with butter culture tended to increase slightly. Development of  $ac_2$  in butter was not retarded, even when the butter was manufactured under reduced air pressure. An increase in  $ac_2$  content of butter on holding 25 days at 40° F. was not considered due to atmospheric oxidation.

Bunger (16) found the  $ac_2$  content of sour cream butter held at 62.6° to 71.6° F. remained practically the same during the first 4 days, whereas hold-

ing at 46.4° to 50° F. gave a slight increase in  $ac_2$ . After 12 days at 46.4° to 50° F., or higher, a decrease was noted in all cases. At 14° to 32° F. the  $ac_2$  content was the same after 10 days.

According to Virtanen (128) the aroma of butter made from aerated cream, as well as that of butter made from non-aerated cream, greatly decreased during storage. Deterioration in butter made from aerated cream was no more extensive than in butter made from non-aerated cream; after 1 month both samples were graded approximately equal. Virtanen stated that disappearance of butter aroma during storage is an important problem for export countries, where butter cannot be sold fresh.

Matuszewski *et al.* (69) noted a distinct decrease and even disappearance of butter aroma in butter held 1 week at 50° to 59° F.; the butter decreased in both  $ac_2$  and  $amc$ . Brioux and Jouis (13) reported that the amount of  $ac_2$  in butter decreased rapidly. Within 15 to 18 days after manufacture, the  $ac_2$  content of butter decreased to about one-tenth of its original value. One lot of butter containing 1.5 mg.  $ac_2$  per kg. the day after churning contained only 0.05 mg. 26 days later.

Results of Davies (24, 25, 26) indicated that ripened cream butter requires from 12 to 24 hours before the full flavor develops. During this period  $amc$  is oxidized to  $ac_2$  and an equilibrium is set up in the ratio of  $ac_2$  to  $amc$  which is different from that in cream. Davies indicated that a different ratio is to be expected because there is practically no oxygen in solution in ripened cream while fresh butter is saturated with air.

Slatter and Hammer (117) did not find a significant increase in  $amc + ac_2$  when salted butter was held at various temperatures. Butter containing 0.75 per cent salt showed a rather rapid disappearance of  $amc + ac_2$  at 44°, 50° or 60° F. The amounts of  $amc + ac_2$  in butter containing 1.0, 1.5 or 2.5 per cent salt remained fairly constant. Failure to obtain significant increases in the  $amc + ac_2$  in salted butter was expected because of the restraining action of salt on the culture organisms.

Data obtained by Brioux and Jouis (14) indicated that in fresh butter  $ac_2$  disappears rapidly when the butter is held. Butter containing 1.50 mg.  $ac_2$  per kg. the day following manufacture, showed 0.60 mg. after 5 days, 0.13 mg. after 18 days and 0.05 mg. after 27 days. The  $amc$  content was 42.4 mg. per kg. the day following manufacture and 20.4 mg. after 27 days.

Pont (94) stated that full flavor does not develop in butter until approximately 24 hours after churning. There was no flavor development in butter held at the freezing point or lower.

Mohr *et al.* (78) found that sweet cream butter, with as much as 0.4 mg.  $ac_2$  per kg. when fresh, contained practically none after 6 months in storage. When sour cream butter containing 1 to 2 mg.  $ac_2$  per kg. was stored 6 months, the  $ac_2$  content was reduced or remained constant. Various packing materials, such as parchment paper, transparent water-proof paper and

glass, had no effect on the  $ac_2$  content. Storage of the butter in an atmosphere of carbon dioxide also had no effect. Iron and copper introduced into the butter from equipment increased the  $ac_2$  content during storage but often caused off flavors.

Analyses on salted butter at various periods by Prill and Hammer (99) showed a rather high retention of  $ac_2$  and  $amc$ , even at 70° F. Development of tallowiness at this temperature, which is common because of the great effect of relatively high temperatures on appearance of the defect, was not accompanied by a sharp decrease in  $ac_2$  or  $amc$  contents.

Toth (125) noted that with butter prepared from acid cream the amount of aroma-producing substances increased for several days and then rapidly decreased. With butter made from sweet cream, a slow but constant decrease was observed; storage at low temperatures impeded these changes.

Hoecker and Hammer (47) obtained both increases and decreases in  $ac_2$  with butter held 1 day at 40° F. and then 2 and 4 weeks at 0° or 35° F., the larger changes usually occurring at 35° F. Occasionally, increases in  $ac_2$  contents after 2 weeks were followed by decreases after 4 weeks. Except in a few instances, the  $amc$  contents did not change appreciably.

**Unsalted butter.** Results obtained by Slatter (115) showed that  $amc + ac_2$  commonly was produced in unsalted butter made with culture when the butter was held under favorable conditions, although the amounts in different lots held under the same conditions varied considerably. The largest production usually occurred at the highest holding temperature (60° F.) and in lots which developed the lowest pH. In several instances the amounts of  $amc + ac_2$  in unsalted butter decreased during the first few days of holding and remained low, while in other cases a decrease was followed by an increase. A decrease regularly followed the maximum production of  $amc + ac_2$ . A larger production of  $amc + ac_2$  occurred in the butter when 10, 15 or 20 per cent butter culture was added to the cream than when 5 per cent was used. In some cases addition of flavor-producing streptococci to the cream along with butter culture appeared to increase production of  $amc + ac_2$  in butter during the holding.

Slatter and Hammer (117) found that when unsalted butter was held at 0°, 34° or 44° F. the amounts of  $amc + ac_2$  sometimes increased and sometimes decreased, both increases and decreases being more definite at 44° F. than at lower temperatures. Increases commonly occurred at 50°, 60° or 70° F. At 50° and 60° F., maximum production usually was noted after 7 days. At 70° F. there was a decrease from the fourth to the seventh day. The pH of butter held at 44°, 50° and 60° F. was reduced gradually while at lower temperatures not much change occurred. A low pH in butter commonly was accompanied by a large production of  $amc + ac_2$ . Lowering of the pH and production of  $amc + ac_2$  at relatively high temperatures sug-

gest that essentially the same changes take place in unsalted butter as in butter culture. The decrease in  $\text{amc} - \text{ac}_2$  in unsalted butter is in agreement with the disappearance of these materials in butter cultures and is due, presumably, to reduction of  $\text{amc}$  or  $\text{ac}_2$  to 2,3-butylene glycol by the flavor-producing streptococci. Development of  $\text{amc} - \text{ac}_2$  at favorable temperatures suggests that a short ripening period may be desirable for commercial unsalted butter from the standpoint of obtaining flavor. However, these temperatures may also favor development of undesirable organisms.

Results of Mohr and Wellm (79) indicated that holding temperature exerts a decisive influence on the  $\text{ac}_2$  and  $\text{amc}$  contents of butter. At  $62.6^\circ$ ,  $64.4^\circ$  and  $71.6^\circ$  F. the amounts of  $\text{ac}_2$  in sour cream butter remained practically constant for the first 4 days. Butter held at  $50^\circ$  F. usually showed an increase after 4 days. The  $\text{ac}_2$  content decreased considerably in butter held 12 days above  $46.4^\circ$  F.; at  $14^\circ$  to  $32^\circ$  F. it remained practically constant, even after 12 days.

Prill and Hammer (99) found significant changes in  $\text{ac}_2$  and in  $\text{amc} - \text{ac}_2$  in unsalted butter held at  $36^\circ$  to  $45^\circ$  F. and at  $70^\circ$  F.; undoubtedly these were due to activity of the culture organisms. The changes involved both increases and decreases, as would be expected from the general relationship of the culture organisms to  $\text{amc}$  and  $\text{ac}_2$ . An increase in the  $\text{ac}_2$  content of unsalted butter was believed to be important in the flavor development which this product often undergoes; a subsequent decrease presumably is accompanied by a partial loss of flavor.

Analyses by Hedrick and Hammer (45) on lots of unsalted butter before and after holding showed the following: Of 56 lots of butter held 1 week at  $36^\circ$  to  $40^\circ$  F., 47 showed increases in  $\text{ac}_2$ , 2 showed no change and 7 showed decreases; with the same lots, 38 showed increases in  $\text{amc}$  and 18 showed decreases. When the lots were held 3 days at  $60^\circ$  F. plus 4 days at  $40^\circ$  F., 45 showed increases in  $\text{ac}_2$  and 11 showed decreases; with the same lots (minus 1), 37 showed increases in  $\text{amc}$  and 18 showed decreases. Of 43 lots of butter held 1 month at  $36^\circ$  to  $40^\circ$  F., 39 showed increases in  $\text{ac}_2$  and 4 showed decreases; with the same lots (plus 1), 29 showed increases in  $\text{amc}$ , 1 showed no change and 14 showed decreases. The  $\text{ac}_2$  and  $\text{amc}$  contents of butter held 3 days at  $60^\circ$  F. plus 4 days at  $36^\circ$  to  $40^\circ$  F. and of butter held 1 week at  $36^\circ$  to  $40^\circ$  F. were compared. The holding at  $60^\circ$  F. resulted in a higher  $\text{ac}_2$  content in 42 of the 56 comparisons, no change in 1 and a decrease in 13; the  $\text{amc}$  content was higher in 40 of the 55 comparisons, the same in 4 and lower in 11.

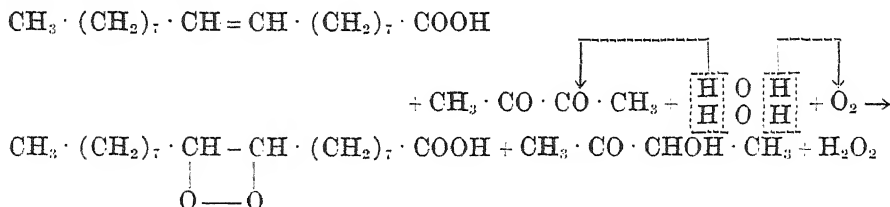
#### ACTION OF ACETYLMETHYLCARBINOL AND DIACETYL ON BUTTER

Recognition of the importance of  $\text{amc}$  and  $\text{ac}_2$  from the standpoint of flavor and flavor development in butter at once suggested consideration of the relationship of these compounds to butter deterioration. Two angles

are involved, chemical and microbiological. Since  $ac_2$  is rather highly oxidized, it readily acts on certain compounds that are easily oxidized and in this way might produce off flavors in butter. Also,  $ac_2$  has germicidal properties and might influence growth of microorganisms in butter, thus preventing development of certain off flavors.

### Chemical Considerations

King (56) studied the influence of  $ac_2$  on butterfat by adding it to melted and filtered fat, obtained from sweet cream, in concentrations of 0.05, 0.01 and 0.005 per cent. The fat used was almost odorless and the  $ac_2$  went into it on warming. One series of tests was conducted in diffuse daylight at approximately  $64.4^\circ$  to  $73.4^\circ$  F. while the other was carried out in the dark at  $71.6^\circ$  to  $75.2^\circ$  F. In the presence of air the butterfat became bleached and tallowy. The change was accelerated by light. Bleaching was first evident at the surface and progressed from there. In general, tallowiness appeared later than the bleaching. Rate of bleaching and degree of tallowiness were proportional to the amount of  $ac_2$  present. King suggested that  $ac_2$  may act on butterfat by oxidizing oleic acid to oleic acid peroxide, which then breaks up into various compounds having a pronounced tallowy odor and flavor, the  $ac_2$  being reduced to amc or even to 2,3-butylene glycol. The following reactions were suggested as explaining the action of  $ac_2$  on oleic acid:



The hydrogen peroxide reacts with hydrogen to form water. Fixation of oxygen on the colored material of the fat also was suggested since the carotinoid pigments are rich in double bonds.

According to Tapernaux (120) the more aroma butter possesses, the poorer is its keeping qualities. Kay (54) reported that oxidation of amc during storage of butter indicates oxidative changes in the butterfat. Butter with pronounced aroma had poorer keeping qualities than butter with less aroma. Davies (22) noted that one of the disadvantages of amc in butter is its action as an oxidation catalyst, accelerating deterioration of the fat.

Hammer (40) stated that  $ac_2$  is highly oxidized and can react with various materials. Large quantities of it bleached butterfat and produced tallowy odors and flavors. Small amounts, such as are present in butter, were not considered a direct cause of these defects. Action of  $ac_2$  was consider-

ably slower if an acid, such as lactic or sulfuric, was present. Hammer noted that  $ac_2$  worked into butter may have a better opportunity to react with fat and other constituents than if it is largely present in the moisture droplets of the butter. Later, in commenting on King's studies (56) Hammer (41) pointed out that butterfat exposed to air develops a tallowy odor and flavor, the action being surprisingly rapid. He also suggested that observations on the action of  $ac_2$  on butterfat cannot be directly applied to the action of  $ac_2$  when it is added to butter for the purpose of improving the flavor because of the very small amounts used and probably for other reasons also.

An article published by the Polaks Frutal Works (93), and intended to refute a published statement (2), indicated that  $ac_2$  does not exert a definite oxidizing action on fats but has a tendency to reduce.

Barnicoat (5) did not consider  $ac_2$  in itself to be responsible for the poorer keeping qualities of ripened cream butter. Later, he (7) stated that  $ac_2$  has no effect on the keeping qualities of butter made from "mild starter" cream. Added  $ac_2$  (6 ppm.) was not important in promoting deterioration of butter held 3.5 months at 14° F. Barnicoat noted that marked deterioration of high acid butter is related to low pH and not to its high  $ac_2$  content.

Results obtained by Davies (23) led to the conclusion that with low-flavored butter it is unlikely the traces of  $ac_2$  (0.1 to 0.3 ppm.) present will initiate autoxidation in the absence of considerable serum acidity; nor is this the case with medium-flavored butter (0.4 to 0.8 ppm.  $ac_2$ ) or full-flavored butter (0.9 to 2.0 ppm.  $ac_2$ ). Much larger concentrations of  $ac_2$  (6.0 ppm.) were very active in promoting fat autoxidation, the compound being more active in this respect than an equivalent amount of oleic acid. Davies also reported (24) that tallowiness in full-flavored butter is not due so much to  $ac_2$  as to conditions associated with manufacture of such butter. Later (25, 26, 27), he stated that full-flavored butter does not keep well but develops fishiness and oxidative rancidity much more rapidly than butter made from sweet or neutralized cream. The aroma constituent was not considered responsible for these defects but rather the high acid content. The amount of  $ac_2$  in butter was regarded as too small to initiate or catalyze oxidative changes.

Pont (94) indicated that the peroxides formed as a result of the liberation of oleic acid oxidize  $ac_2$  to flavorless compounds during butter deterioration. Further changes involve breakdown of lecithin, resulting in fishy flavor, and oxidation of butterfat, resulting in stale and tallowy flavors.

The peroxide numbers on samples of butterfat which were heated to 219.2° F. and held there for 8 hours were determined by Ritter and Nussbaumer (101). Addition of 0.1 or 1 per cent  $ac_2$  to the butterfat resulted in a significant increase in peroxide number; the addition decreased the oxidation resistance of the butterfat. When butterfat contained 0.1 or 1

per cent added  $ac_2$ , it was much more susceptible to oxidation than control samples or samples containing less than 0.01 per cent  $ac_2$ . The greater the susceptibility of untreated butterfat to oxidation, the greater the accelerating influence of  $ac_2$ .

Wiley (136) stated that the presence of butter culture organisms in butter favored oxidation of the fat at cold storage temperatures. This was thought to be due to some product of bacterial metabolism acting as a pro-oxidant; the product was not  $amc$  or  $ac_2$ .

In referring to the work of King (56) Prill and Hammer (99) noted that he used pure butterfat with a relatively high concentration of added  $ac_2$  and, in some cases, rather drastic treatments, such as exposure to light, while in butter it is probable that much of the  $ac_2$  is in the water phase rather than entirely in the fat. In general, it appeared that the amount of  $ac_2$  ordinarily encountered in butter has no significant effect on promotion of chemical defects and that other factors, such as copper content of the butter and pH of the serum, are of more importance in this connection.

Barnicoat (7) compared the vitamin A and carotene contents of lots of butter (made from portions of the same batch of cream) after 22 months at  $14^{\circ}$  to  $17^{\circ}$  F.;  $ac_2$  was added to two of the lots during working at the rate of 4 ppm., or more than 12 times the concentration usually found in "mild starter" butter. The loss of vitamin A and its precursor carotene in both sweet cream and "mild starter" butter containing unusually large proportions of  $ac_2$  was not greater than in control butter churned from sweet cream.

Hunziker and Cordes (48; p. 357) studied several thousand churnings of butter made from unripened cream in which  $ac_2$  (approximately 3 to 4 ppm.) was added to the butter. After holding the butter 2 to 9 months, there was no indication of bleaching, tallowy flavors or other flavors such as might be attributable to oxidation by  $ac_2$ .

Toth (125) stated that too high a concentration of  $ac_2$  is disadvantageous in the storing of butter since, on its reduction to  $amc$ , fats become rancid.

### *Microbiological Considerations*

Lemoigne (61) stated that  $ac_2$  in relatively small and variable amounts can retard development of micro-organisms. Experiments of Lévy-Bruhl and Cado (62) showed that *Staphylococcus aureus* was killed in bouillon by 0.1 per cent  $ac_2$  but not by 0.04 per cent. Various organisms, including streptococci, pneumococci, gonococci and typhoid, paratyphoid, diphtheria and coliform types, were killed by 0.04 per cent but not by 0.02 per cent.

### AMOUNTS OF ACETYLMETHYLCARBINOL AND DIACETYL IN VARIOUS DAIRY PRODUCTS

Over the years in which there has been an active interest in  $amc$  and  $ac_2$  from the standpoint of flavor development in butter and related prod-



ucts, the methods of determination have changed greatly. For the detection of relatively small amounts of these compounds, colorimetric methods now are commonly used, instead of the less sensitive gravimetric methods (43; p. 156). This change in analytical procedure should be recognized in any consideration of the reported  $\text{amc}$  and  $\text{ac}_2$  contents of dairy products.

### Milk

Testoni and Ciusa (123, 124) did not find  $\text{ac}_2$ ,  $\text{amc}$  or 2,3-butylene glycol in fresh milk; Mohler and Herzfeld (76) and also Pien *et al.* (91) did not detect  $\text{ac}_2$  in it. Schmalfuss and Werner (112) reported that milk intended for household use contained no detectable amounts of  $\text{ac}_2$  or  $\text{amc}$ , that is, less than 0.000024 per cent.

$\text{Ac}_2$  was not found in spontaneously soured milk by Testoni and Ciusa (123); later studies (124) indicated that old milk may contain some 2,3-butylene glycol. Schmalfuss and Barthmeyer (111) detected both  $\text{ac}_2$  and  $\text{amc}$  in sour milk, about ten times more  $\text{amc}$  than  $\text{ac}_2$  being present. Pien *et al.* (91) did not find  $\text{ac}_2$  in acid coagulated milk.

### Skimmilk

When 1 l. was used for the distillation, Krenn (58) did not find  $\text{ac}_2$  in skimmilk. Schmalfuss and Werner (112) reported neither  $\text{ac}_2$  nor  $\text{amc}$  in fresh skimmilk. Mohr and Wellm (79) believed that skimmilk contains both  $\text{ac}_2$  and  $\text{amc}$ , although only to a very small extent (0.17 mg.  $\text{ac}_2$  per l.).

### Cream

Testoni and Ciusa (123) detected  $\text{ac}_2$  in centrifuged cream. According to Michaelian and Hammer (73) sweet cream frequently contains a small amount of  $\text{amc} + \text{ac}_2$ ; however, cream freshly skimmed from milk low in bacteria showed none in 200 g. Pien *et al.* (91) found no  $\text{ac}_2$  in fresh cream. Slatter (116) stated that since a high acidity is necessary for production of  $\text{ac}_2$ , it is not found in sweet cream. Krenn (58) did not detect  $\text{ac}_2$  in sweet cream when 1 l. was used for the distillation.

Tapernaux (120) reported that  $\text{ac}_2$  can be detected in ripened cream, especially when the ripening is not preceded by pasteurization. According to Virtanen and Tarnanen (129) ripened cream contains practically no  $\text{ac}_2$  but considerable amounts of  $\text{amc}$ . Pien *et al.* (91) obtained variable results when testing spontaneously soured cream for  $\text{ac}_2$ ; the samples always showed some  $\text{ac}_2$  (about 1.5 mg. per l.). Cream several weeks old showed about 1.3 mg. per l. Schmalfuss and Werner (112) found the  $\text{ac}_2$  content of sour cream to be 0.000024 per cent. Slatter (116) stated that only small amounts of  $\text{ac}_2$  are found in sour cream.

Additional data on the  $\text{amc}$  and  $\text{ac}_2$  contents of cream intended for butter are given in the consideration of the influence of cream acidity on the  $\text{amc}$  and  $\text{ac}_2$  contents of butter.

### Other Products

Pien *et al.* (91) did not detect  $ac_2$  in aqueous solutions of lactic acid from fermented milk. Considerable quantities of lactic acid gave a slight test for  $ac_2$  and the distillate from 25 ml. syrupy lactic acid gave a positive reaction corresponding to 0.3 mg.

Schmalfuss and Werner (112) obtained a positive test for  $ac_2$  on the material recovered by dry distillation of milk sugar. Dry distillation of citric acid did not give a positive test. The authors (112) found the  $ac_2$  content of commercial margarine to be 0.00014 per cent.

### Butter

van Niel *et al.* (126) found from 0.0002 to 0.0004 per cent  $ac_2$  in fine butter. Schmalfuss and Barthmeyer (110) analyzed four samples of butter representing different conditions of feeding the producing animals and obtained the following weights of nickel salt (43; p. 156) equivalent to  $ac_2$  per kg.: 0.0006, 0.0002, 0.0003 and 0.0001 g. The quantity of  $ac_2$  in the butter appeared to be correlated with the intensity of the aroma. Testoni and Ciusa (122) stated that melted and pasteurized butter, as well as margarine, do not contain  $ac_2$ , whereas fresh butter contains about 0.00051 per cent. They believed the presence of  $ac_2$  was a measure of the freshness of the butter. Later (123), they did not find  $ac_2$  in properly prepared butter.

According to Tapernaux (120) fresh unpasteurized butter contains from 0 to 50 g.  $ac_2$  in 100 kg.; pasteurized butter contained traces. Using a method capable of detecting 0.02 or more g.  $ac_2$  per kg., Vizern and Guillot (130) obtained negative tests on butter. Hammer (40) found more amc than  $ac_2$  in butter made with butter culture. Davies (22) stated that butter made from ripened cream may contain 0.05 to 0.5 ppm. of  $ac_2$ . Mohler and Herzfeld (76) reported that butter of normal aroma contains 0.0002 to 0.0004 per cent  $ac_2$ .

Michaelian and Hammer (73) examined 56 lots of butter for amc +  $ac_2$ . With a gravimetric method, 4 of them showed none while the remaining 52 yielded from 0.1 to 3.45 mg. nickel salt per 200 g. Steuart (118) found that the quantity of  $ac_2$  in butter ranged from 0 to 5 ppm., depending on the presence and proportion of aroma bacteria in the souring cream. Testoni and Ciusa (124) stated that Italian butters do not normally contain  $ac_2$ ; its presence was regarded as accidental. Pien *et al.* (91) found very little or no  $ac_2$  in good butter (trace to 1 mg. per kg.).

Barnicoat (7) reported no correlation between the  $ac_2$  content of butter and the score. Butter made with culture, which was quite acceptable to the graders, generally contained 0.2 to 0.4 ppm.  $ac_2$  and 1 to 3 ppm. amc. "Mild starter" butter contained about eight times the  $ac_2$  in sweet cream butter, but only one-fourth of that in highly flavored butter.

Of the butter samples analyzed by Brioux and Jouis (13), 76.3 per cent contained  $ac_2$  in amounts ranging from a trace to 0.5 mg. per kg.; the re-

mainder contained from 0.51 to 1.5 mg., except in one instance for which the value was 2.5 mg. The amc contents were much higher, usually from 10 to 30 mg. per kg., with a maximum of 69 mg.

Davies (24) reported that the absence of  $ac_2$  gives a flavorless butter, 0.2 to 0.6 ppm. gives a mild flavor and 0.7 to 1.5 ppm. gives a full flavor; 2 ppm.  $ac_2$  gives a strong flavor and aroma which is harsh and repulsive to butter graders. Makar'in (67) stated that the aroma of butter is pronounced with an  $ac_2$  content of 0.00048 per cent in the plasma. An  $ac_2$  concentration as low as 0.0013 per cent produced an odd, sharp odor.

According to Matuszewski *et al.* (69) butter made from cream that had been inoculated with a culture of *S. diacetylactis* contained about 5 mg. nickel salt per kg.

Mohr and Wellm (79) noted that the method of producing butter, whether from sweet or sour cream, is of major importance with regard to the  $ac_2$  content. Sweet cream butter contained considerably less  $ac_2$  than sour cream butter. As much as 0.2 mg.  $ac_2$  per kg. and 0.36 mg. amc +  $ac_2$  was found in sweet cream butter. In various lots of German first-class butter, the  $ac_2$  content varied from 0.34 to 1.66 mg. per kg. and the amc +  $ac_2$  content from 3.73 to 20 mg.

Of the 130 samples of butter investigated by Pien *et al.* (92), 11 per cent contained  $ac_2$  in amounts ranging from 0.5 to 1.0 mg. per kg., 47 per cent contained from 0.1 to 0.5 mg. and 42 per cent contained less than 0.1 mg.

Schmalfuss and Werner (112) found the  $ac_2$  content of German butter made in August to be 0.00003 per cent. Slatter and Hammer (117) reported that the amount of amc +  $ac_2$  in butter at the time of churning varied with the amount of butter culture used. Dehove and Dessirier (29) stated that all butter naturally contains a small amount of amc.

Brioux and Jouis (14) examined samples of fresh butter and found that they normally contained a small quantity of  $ac_2$ , usually from 0.05 to 0.5 mg. per kg.; in rare cases as much as 2.5 mg. was found. Butter made in cooperative or industrial creameries contained considerably more  $ac_2$  than butter made on farms. The amc contents were considerably higher than the  $ac_2$  contents; usually butter contained between 10 and 30 mg. per kg. but some lots contained as much as 69 mg. Amc did not appear to give rise to an appreciable quantity of  $ac_2$ .

Alberti (1) found the amount of  $ac_2$  in butter varied from a trace to 0.4 mg. per kg. when the samples were from the same source. The amc contents were from 10 to 40 times the  $ac_2$  contents.

According to Mohr *et al.* (78) fresh sweet cream butter contained up to 0.4 mg.  $ac_2$  per kg. and sour cream butter from 1 to 2 mg. Salted and unsalted butter showed no difference in  $ac_2$  contents.

In comparisons involving addition of equal percentages of regular and aerated or modified aerated (p. 88; p. 89) culture to pasteurized sweet cream,

Prill and Hammer (98) noted that butter made with the latter types of culture invariably had the higher  $ac_2$  contents (0.38 to 1.0 mg. per kg. compared to 0.14 to 0.38 mg.). However, the higher values were much lower than they would have been if the  $ac_2$  contents of the butter were proportional to those of the cultures.

Toth (125) found that Hungarian butter contained 0.05 to 1.5 mg. per cent  $ac_2$  and 1.5 to 4.2 mg. per cent  $ame$ .

Hoecker and Hammer (47) noted that butter made without butter culture or with *S. aromaticus* contained only small amounts of  $ac_2$  and  $ame$ , whereas butter made with butter culture, *S. citrovorus*, *S. paracitrovorus*, *S. diacetylactis*, *S. citrophilus* or an unidentified organism commonly contained appreciable amounts.

#### BUTTER CULTURE DISTILLATE AS A SOURCE OF FLAVOR FOR BUTTER

$Amc$  in various materials is readily converted to  $ac_2$  by oxidation with some such reagent as ferric chloride, and the  $ac_2$  thus formed, as well as that originally present, can be distilled out. Distillates obtained from butter culture with this general procedure (often called starter distillates) have been suggested for producing flavor in butter, the distillates being worked into the butter during the normal working process. Because a butter culture contains much more  $ame$  than  $ac_2$ , a distillate contains much more  $ac_2$  than the volume of culture from which it was obtained. However, some of the  $ame$  distills over before its conversion to  $ac_2$  (72) and appears as such in the distillate.

Since volatile acids, and possibly other volatile compounds, contribute to the flavor of butter culture, a culture distillate may contain materials other than  $ac_2$  that are desirable from the standpoint of butter flavor. On the basis of the present knowledge, volatile acids appear to be of most importance in this connection.

Ruehe and Ramsey (107) noted that the flavor constituents of butter cultures, including  $ac_2$  and  $ame$ , are removed by steam distillation. Addition of the distillate to sweet cream before churning yielded butter of intensified aroma. Similar results were obtained by adding the distillate directly to butter. Butter cultures used for steam distillation were grown in such a manner that they contained increased amounts of the flavoring constituents.

Use of an  $ac_2$  concentrate was suggested by Davies (23). The concentrate was prepared by distillation of  $ac_2$  from butter culture and addition of the distillate to neutralized butter culture. Such preparations contained 300 times more  $ac_2$  than ripened cream.

According to Ruehe (105) the  $ac_2$  content of butter culture distillate does not change appreciably, even after 63 days at 40° F. When butter was made with various amounts of distillate, most judges preferred that containing less than 1 part  $ac_2$  in 200,000 parts of butter.

The scheme suggested by Ruehe (106) for controlling flavor in butter consists of selection of cultures which actively produce lactic acid and ferment citrates when grown in milk, adoption of methods which are conducive to high yields of amc, steam distillation of the culture, standardization of the  $ac_2$  content of the distillate and addition of the distillate to the churned butter at the time of salting. Advantages of the suggested method are that the distillate can be prepared in the laboratory and supplied to various plants, flavor intensity can be adjusted to demands of the trade, high-flavored butter with good keeping qualities can be produced, butter has a low bacterial count and culture distillate is more economical than butter culture. In a series of comparisons involving use of 2.5 per cent butter culture and various amounts of  $ac_2$  as butter culture distillate, the butter containing  $ac_2$  as distillate in the ratio of 1 part  $ac_2$  to 400,000 parts of butter scored highest when fresh; after 2 months at 40° F., the butter containing  $ac_2$  as distillate in the ratio of 1 part  $ac_2$  to 800,000 parts of butter scored highest. Similar results were obtained when the lots of butter were held at -10° F. for 8 months and scored at intervals of 2 months.

#### DETECTION OF ADDED DIACETYL IN BUTTER.

In the examination of butter, detection of  $ac_2$  added as such, or in the form of a flavor concentrate, sometimes is attempted. It is complicated by the fact that  $ac_2$  commonly is present in butter through use of butter culture and/or cream containing  $ac_2$ .

Davies (22) noted that a relatively high  $ac_2$  content and a low acidity<sup>1</sup> in butter should indicate that  $ac_2$  had been added artificially. Later, he (27) reported that analytical evidence for detection of added synthetic  $ac_2$  in butter is unsatisfactory. The two main evidences of such addition are acidity of the butter serum and ratio of  $ac_2$  to amc. The author stated that butter serum having a pH of 6.4 to 6.8 should not contain a detectable amount of  $ac_2$ . The ratio of  $ac_2$  to amc in normal butter was considered to be from 1:15 to 1:20. Butter culture distillates had ratios of 5:1 to 2:1.

The ratio of  $ac_2$  to amc in butter was suggested by Barnicoat (5) as of value in the detection of added  $ac_2$ . Bacteriological examination was proposed as a guide for determining whether butter culture had been used in the cream from which the butter was churned.

Pien *et al.* (92) stated that since none of the butter examined contained more than 1.0 mg.  $ac_2$  per kg., samples containing larger amounts had  $ac_2$  added to them artificially.

Hoecker and Hammer (47) studied pure milk cultures of various streptococci, including *S. citrovorus*, *S. paracitrovorus*, *S. diacetylactis*, *S. citrophilus*, an unidentified organism and *S. aromaticus*. With each species the ratio of  $ac_2$  to amc varied in the different trials; frequently the  $ac_2$  was much higher in proportion to the amc than with butter cultures. The results

suggest the dangers in using the  $ac_2$  to amc ratio in detecting added  $ac_2$  in butter.

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## A SATISFACTORY METHOD OF SHIPPING DAIRY BULL SEMEN LONG DISTANCES\*

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The methods of collecting, storing, and handling bull semen after collection have been widely investigated in recent years for the purpose of extending the use of valuable sires through artificial insemination (1, 3, 5, 6). Satisfactory progress has been made in this field especially in its application to large cooperative associations where the bulls are within easy reach of the cows to be inseminated. It is often desirable, however, to breed certain cows to bulls which are a great distance away. Shipment by the ordinary means of transportation available may require two days or more in many instances. This situation presents a different transportation and storage problem than is encountered in shorter shipping distances.

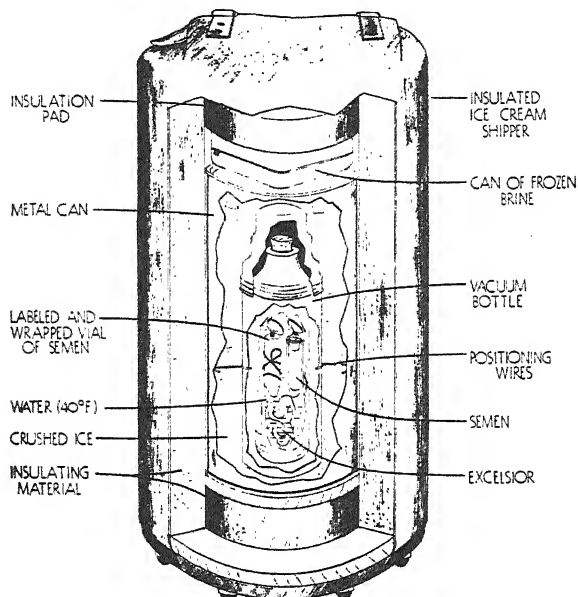
Numerous investigators (1, 2, 3, 5, 6) have shown that the proper temperature for storage of bull semen is below 45° F. and preferably at or slightly below 40° F. or 5° C. Hence, some reliable practical method of refrigeration and temperature control is necessary. Glass vacuum bottles alone may be used, but in addition to being very fragile, they are unsuited for lengthy shipment because of the relatively short period at which a temperature as low as 40° F. may be maintained. A light cardboard shipping container, described by Salisbury (4) has proved suitable in artificial breeding association work where the shipping time does not exceed 24 hours. Ice-filled thermal jugs likewise have been used successfully for short-time shipment. In addition to providing only a short safe shipping time, these types of packages have given difficulty because the semen is in close contact with ice which in cold weather may cause the temperature of the semen to drop too low. An investigation was therefore conducted to find a shipping method that could be depended upon to maintain proper refrigeration for 48 hours or more and at the same time not harm the semen by too low temperatures. A prime consideration was to adopt a container which would withstand the rigors of shipping. The type of package finally adopted and preliminary results from its use are reported below.

The requirements of long distance shipping were; 1) adequate refrigeration of the package, 2) protection of the semen from the intense cold of the refrigerant, and 3) insulation of the refrigerant against the external temperature. It was desired to meet these requirements as simply as possible so that the method would be quickly and easily available to all who wished

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to use it. For this reason utilization was made of existing equipment entirely. The refrigerant used was mainly crushed ice with a can of frozen brine used for extra hot weather. A small vacuum bottle filled with water at 40° F. was used to contain the vials of semen; and commercially available small insulated shipping packages such as are commonly used for ice cream were used as external insulation. These were combined as diagrammed in figure 1.



### A PACKED SEMEN SHIPPER

Fig. 1. A cut-away diagram of the packed semen shipper.

The cooled or partially cooled semen in an insulated, rubber-wrapped vial was placed in a vacuum bottle of water at 40° F. A few strands of excelsior in the bottle prevent undue bobbing of the vials during transit. The vacuum bottle, with supporting wires to position it at the center, was put into a metal can and chipped ice was packed around it as shown in figure 1. The can was sealed and placed in a small ice cream shipper. Best results were secured when the can fit in the shipper tightly or was packed in with insulating material. If extra refrigeration is desired a can of frozen brine may be placed on top of the can. The shipper should be packed quickly, closed, and shipped. The packed shipper weighs between 15 and 20 pounds, depending upon how much ice is used; so it can be easily shipped by express or parcel post.

This shipper has maintained a satisfactory semen storage temperature for more than 84 hours at summer temperatures (80° F.) and several ship-

ments (two in hot August weather) have been successfully made in which the package was three days in transit. Maximum holding time can be obtained by precooling the shipper and using a maximum of ice plus the frozen brine pad.

The semen is protected from the severe cold of the ice by means of the water in the vacuum bottle. The size of the vacuum bottle and the amount of ice used can be varied for different outside temperature conditions. In summer shipments a one-half pint vacuum bottle provided enough water insulation so that the temperature of the semen did not fall below  $34^{\circ}$  F. Also, a large amount of ice could be packed around this small bottle. In cold winter weather, however, only a small amount of ice was needed in the can to insure proper refrigeration. A full can of ice around a one-half pint bottle was not satisfactory with the outside temperature below  $32^{\circ}$  F. because the temperature of the semen dropped to  $32^{\circ}$  F. Three different shipments of semen known to be fertile under proper storage conditions produced no pregnancies when they had been cooled to  $32^{\circ}$  F. By using a pint bottle in place of the one-half pint bottle and packing the can only half full with crushed ice, the temperature of the water did not fall below  $34^{\circ}$  F. and the fertility of the shipped semen was maintained very satisfactorily. Therefore, the recommended procedure is to use a pint vacuum bottle and a small amount of ice in winter conditions and to use a one-half pint bottle with a maximum amount of ice in hot summer conditions.

The glass vacuum bottles in these containers are subject to shattering under very rough handling. With ordinary care and handling, however, no breakage has occurred. If breakage of the bottle does occur, the chances are still good for the semen to come through in satisfactory condition because of the refrigerant being outside of the bottle.

Twenty-four shipments of semen have been made by this method in the course of twelve months' investigations. When the proper precautions as outlined above have been observed, the shipments have been successful in delivering semen with motility as good or better than that obtained from the same semen stored at  $40^{\circ}$  F. in a refrigerator. Furthermore, the few inseminations made have yielded satisfactory results. Eight conceptions have been secured from twelve inseminations using semen ranging from 48 to 130 hours of age regardless of its motility rating. The semen used ranged from 70 to 90 per cent motility when packed and was from University of Missouri bulls known to have a low insemination-per-conception ratio. The inseminations made with the semen which was cooled to  $32^{\circ}$  F. in transit were not included in this summary. Six inseminations which were made with semen which had been stored 144 hours or more (all of very poor motility) were infertile. Results have been, therefore, such as would be expected from the use of properly handled stored semen. The success in shipping semen by the method described will depend largely upon the ability of the semen to maintain fertility during storage at  $35$  to  $45^{\circ}$  F.

## SUMMARY

A simple, dependable, and practical method of shipping semen long distances is described. The package consists of a small insulated ice cream shipper, a water-tight metal can to fit the shipper, crushed ice, and a vacuum bottle. Satisfactory semen storage temperatures (35 to 50° F.) were maintained in this shipper for 84 hours at atmospheric temperature of 80° F. Results of inseminations with shipped semen requiring 48 to 72 hours in transit were very satisfactory for stored semen.

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# THE ACCURACY OF THE MOJONNIER METHOD FOR ESTIMATING MILK FAT IN MILK AND CREAM

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Laboratory technicians have inquired about the degree of accuracy that can be achieved in estimating milk fat in milk by the Mojonnier method. They have also questioned the relative accuracy of single or duplicate determinations on individual samples. Data are presented for milk and cream to answer these questions.

## LITERATURE REVIEW

The literature related to this study is the result of comparing the accuracy of the various volumetric methods with the Røese-Gottlieb method and its mechanized modification, the Mojonnier method, for estimating milk fat in milk and cream. The literature is cited if the data are complete and sufficient to make a contribution of statistical value.

Phillips (4) estimated 50 samples of unpreserved milk in duplicate for milk fat. The individuals in 29 duplicates agreed, 15 differed by 0.01 per cent, 5 by 0.02 per cent and 1 by 0.03 per cent milk fat. Dahlberg, Holm and Troy (1) made 55 estimations for milk fat in duplicate. Some of the estimations were made on replicate samples in different laboratories by the Røese-Gottlieb and the Mojonnier methods. Of the 55 estimations, 14 agreed, 8 differed by 0.01, 18 by 0.02, 9 by 0.03, 1 by 0.04, 2 by 0.05, 1 by 0.07, 1 by 0.22 and 1 by 0.29 per cent milk fat. Fisher and Walts (2) made 32 estimations and approximately 81 per cent of the individuals in duplicates did not differ by more than 0.04 per cent milk fat.

## METHODS

The methods of preparing the samples and estimating them for milk fat have been described (3). After preparing the samples of milk and cream, they were transferred directly to the extraction flasks and weighed (3). Four technicians participated in this study.

It was necessary to obtain some information on the accuracy of estimating milk fat in replicate samples. One hundred estimations were made on 100 samples of the same milk that was produced during the month of June. These individual samples were prepared by agitating 40 quarts of fresh, raw milk at 8–10° C. for about two minutes. As the milk was being

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decrease in milk fat when the samples are held for longer periods, but an observation will be mentioned. Samples 81-88, inclusive, were prepared and weighed into the extraction flasks at the end of the second day. Due to the late hour, the flasks containing the milk were allowed to remain in the laboratory over night and the ether extractions were completed the next day. No sour odors could be detected from these 8 samples the next morning. These samples (81-88) averaged 3.68 per cent milk fat, which is significantly lower than the average for the 100 samples. The other 17 samples in the same group averaged 3.71 per cent milk fat.

The frequency distribution in table 2 shows that 82 of the estimations vary from the mean by 0.02 per cent or less and 18 by more than 0.02 per cent milk fat. Therefore, the odds are 82 to 18 or 4.56 to 1 that individual

TABLE 2  
*Frequency distribution of estimations for milk fat on 100 replicate samples of unpreserved milk*

Per cent milk fat	Frequency
3.66	2
3.67	2
3.68	5
3.69	3
3.70	6
3.71	19
3.72	24
3.73	23
3.74	10
3.75	4
3.76	2
	<hr/> 100

Mean = 3.72 per cent.

Standard deviation = 0.02 per cent.

estimations will not vary from the mean by more than about 0.02 per cent and will agree within 0.04 per cent milk fat. Furthermore, 76 per cent of the estimations are in the frequency groups of 3.71 to 3.74, a difference of 0.03 per cent milk fat.

The results in table 2 should serve as a basis to predict the accuracy that may be attained in routine analyses. To test the validity of this hypothesis, the differences between individuals for each of 341 duplicate estimations on fresh milk were calculated. These estimations were made at regular intervals over a period of about two years. The results are given in the third column of table 3. It is evident that nearly 77 per cent of the individuals in duplicates agreed within 0.03 per cent milk fat and approximately 86 per cent of them within 0.04 per cent milk fat; this agrees closely with the results in table 2.

To show the comparative accuracy of single and of duplicate estimations, it is assumed that the average of duplicates will give the most accurate

results. The variation of the individuals from their mean is one-half of the difference between single determinations for each sample of milk. These calculations were made on the same 341 samples of milk and the results are given in the fourth column of table 3. Eighty-six per cent of the individuals in duplicates did not vary from their means by more than 0.02 per cent milk fat, about 94 per cent by not more than 0.03 per cent milk fat and about 98 per cent by not more than 0.04 per cent milk fat.

Bimonthly composite milk samples preserved with bichloride of mercury were estimated for milk fat in duplicate. These samples were obtained from two milk plants during the summer months. Sixty samples were obtained

TABLE 3

*Frequency distribution of differences between the individuals in duplicates and between individuals and the means of their duplicates for milk fat estimations on 341 samples of unpreserved milk*

Frequency	Per cent	Differences between individuals	
		Duplicates	Means of duplicates
		Per cent milk fat	
70	20.53	0.00	0.000
94	27.57	0.01	0.005
63	18.48	0.02	0.010
36	10.56	0.03	0.015
32	9.38	0.04	0.020
16	4.69	0.05	0.025
12	3.52	0.06	0.030
6	1.76	0.07	0.035
6	1.76	0.08	0.040
2	0.59	0.09	0.045
1	0.29	0.10	0.050
1	0.29	0.11	0.055
1	0.29	0.12	0.060
1	0.29	0.13	0.065
341	100.00		

from Plant A. These samples were in excellent physical condition because they had been properly refrigerated and cared for in the milk plant during the 15-day period. Another set of sixty samples was obtained from Plant B. These samples were not in good physical condition. Some of them showed varying degrees of fat destabilization and mold growth. The results in table 4 show that 95 per cent of the individuals in duplicate estimations from Plant A agreed within 0.03 per cent milk fat and all of them within 0.05 per cent; furthermore, the differences between individual estimations and the means of their duplicates on all the samples are less than 0.03 per cent milk fat. As might be expected, the variability of the results was greater from Plant B, about 73 per cent of the individuals agreeing within 0.03 per cent milk fat and 85 per cent within 0.04 per cent. The increased variability of the results from Plant B can be attributed to the difficulty of obtaining a

TABLE 4

*Frequency distribution of differences between individuals in duplicate estimations for milk fat on 120 samples of preserved milk*

Differences milk fat per cent	Plant			
	A		B	
	Frequency	Per cent	Frequency	Per cent
0.00	13	21.67	6	10.00
0.01	19	31.67	19	31.67
0.02	19	31.67	8	13.33
0.03	6	10.00	11	18.33
0.04	.....	.....	7	11.67
0.05	3	5.00	2	3.33
0.06	.....	.....	2	3.33
0.07	.....	.....	.....	.....
0.08	.....	.....	3	5.00
0.09	.....	.....	2	3.33
	60	100.01	60	99.99

representative sample, because of the destabilized condition of the fat emulsion. The technician who made the estimations on the preserved samples was able to achieve greater accuracy than any of the other three who participated in this work.

Two hundred and forty-two samples of unpreserved cream containing 20–45 per cent of milk fat were estimated for milk fat in duplicate at regular intervals over a period of about two years. It is evident from table 5 that approximately 86 per cent of the individuals in duplicate estimations agreed within about 0.30 per cent milk fat. Approximately 86 per cent of the individuals in the duplicates did not vary more than 0.15 per cent milk fat and about 97 per cent not more than 0.30 per cent from their means.

TABLE 5

*Frequency distribution of differences between the individuals in duplicates and between individuals and the means of their duplicates for milk fat estimations on 242 samples of unpreserved cream*

Frequency	Per cent	Differences between individuals	
		Duplicates	Means of duplicates
		Per cent milk fat	
103	42.56	0.0–0.09	0.0–0.045
68	28.10	0.1–0.19	0.05–0.095
38	15.70	0.2–0.29	0.10–0.145
8	3.31	0.3–0.39	0.15–0.195
12	4.96	0.4–0.49	0.20–0.245
7	2.89	0.5–0.59	0.25–0.295
3	1.24	0.6–0.69	0.30–0.345
1	0.41	0.7–0.79	0.35–0.395
1	0.41	0.8–0.89	0.40–0.445
1	0.41	0.9–0.99	0.45–0.495
242	99.99		

## DISCUSSION

An accuracy can be achieved with single estimations on normal milk samples that may not vary more than 0.03 per cent milk fat in at least 75 per cent of the cases and 0.04 per cent milk fat in at least 82 per cent of the cases (tables 2, 3). An accuracy can be achieved with duplicate estimations that may not vary more than 0.02 per cent milk fat in about 86 per cent of the cases, 0.03 per cent milk fat in about 94 per cent of the cases, and 0.04 per cent milk fat in about 98 per cent of the cases (tables 3, 4).

For cream, approximately 86 per cent of the individuals in duplicate estimations did not vary more than 0.29 per cent milk fat, and about 86 per cent of the individuals in duplicates did not vary more than 0.145 per cent milk fat from their means.

The comparative accuracy of the Mojonnier method on milk and cream can be calculated. Assuming that cream contains 40 per cent milk fat, then the variation between individuals in 86 per cent of the duplicate estimations would be 0.725 per cent of the total milk fat ( $0.29 \div 0.40 \times 100 = 0.725$ ). Using this same method of calculating and assuming that milk contains 4 per cent of milk fat, then the variation between individuals in at least 82 per cent of the cases would be one per cent of the total milk fat ( $0.04 \div 4 \times 100 = 1$ ). Therefore, a slightly higher degree of accuracy can be achieved on cream than on milk.

The condition of the fat emulsion affects the degree of accuracy that can be achieved. Approximately 73 per cent of the individuals in duplicates agreed to 0.03 per cent milk fat from Plant B (table 4) as compared to 95 per cent of the individuals from Plant A. The fat emulsion in the samples from Plant B showed varying degrees of destabilization. Duplicate estimations should be made on milk samples if the fat emulsion is abnormal. For commercial and routine analyses, single estimations on individual samples of normal milk may give sufficient accuracy for practical purposes; however, when a high degree of accuracy is desired, duplicate estimations should be made. The importance of the results to be achieved should determine whether single or duplicate estimations for milk fat are to be made on samples of milk and cream.

## CONCLUSIONS

1. Single estimations of the milk fat content of milk by the Mojonnier method can be expected to give an accuracy within 0.03 and 0.04 per cent milk fat in at least 75 and 82 per cent, respectively, of the cases.
2. The mean of duplicate estimations can be expected to give an accuracy within 0.02 and 0.03 per cent milk fat in approximately 86 and 94 per cent, respectively, of the cases for normal milk.
3. Single estimations of the milk fat content of normal cream by the Mojonnier method can be expected to give an accuracy of about 0.30 per cent milk fat in about 86 per cent of the cases.

4. Duplicate estimations for normal cream can be expected to give an accuracy within 0.30 per cent milk fat in about 97 per cent of the cases.

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# THE MILK AND BUTTERFAT PRODUCTION RESPONSES TO SHARK LIVER OIL IN THE RATION\*

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Reports on the results of feeding shark liver oil to dairy cattle are conflicting.

Deuel and co-workers (1, 4) reported that the daily feeding of 30 to 60 cc. of shark liver oil increased the milk and butterfat production of Guernsey cows approximately 10 per cent. This amount of shark liver oil furnished from 700,000 to 1,400,000 I.U. of Vitamin A.

Rupel, Boyer and Phillips (8) found that the daily feeding of 25 cc. of shark liver oil with a Vitamin A potency of 7,500 I.U. and 15,000 I.U. per gram had no effect on the milk production and fat percentage of Holstein and Guernsey cows. These authors reported that the daily feeding of 90 cc. of a 15,000 I.U. shark liver oil caused a noticeable drop in the amount of 4 per cent fat-corrected milk.

More recently Jensen *et al.* (5) reported that daily feeding of shark liver oil to furnish from 117,500 to 1,350,000 I.U. of Vitamin A resulted in no increase in milk or butterfat production of Jersey, Guernsey, Holstein, and Brown Swiss cows. It was found that the daily feeding of as much as 90 cc. of shark liver oil tended to increase the normal rate of decline in milk production with the advance of lactation.

In view of these conflicting reports the results presented in this paper are offered as additional information.

## EXPERIMENTAL

Twelve cows, 6 Jerseys and 6 Holsteins, were used in the experiment. These cows were divided into 4 groups; group I, Holstein supplement, group II Jersey supplement, group III, Holstein control and group IV, Jersey control. The groups were so divided that the production of each was approximately that of its control group.

All groups were fed the same basal ration, consisting of alfalfa hay, grain mixture and sunflower silage, which was replaced by sweet clover silage after 8 weeks. The grain mixture consisted of barley, oats, wheat bran, linseed oil meal, cottonseed meal, dried molasses beet pulp, iodized salt, and steamed bone meal.

All groups were fed the basal ration during a preliminary period of 4 weeks. Beginning with the fifth week the rations of groups I and II were

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supplemented with 60 cc. daily of shark liver oil with a Vitamin A potency of 25,000 I.U. per gram.

The duration of the experiment was 16 weeks, beginning on January 3, 1942, and ending April 30, 1942.

The shark liver oil supplement was discontinued April 18, 1942, but the cows were kept under observation until April 30, 1942.

Daily milk weight records were kept on all cows during the preliminary and the oil feeding periods. Composite milk samples were taken daily from each cow and the per cent butterfat determined by the Babcock method. Once a week an additional composite milk sample was taken from each cow for Vitamin A and carotene determination.

The butterfat was extracted from the milk and treated prior to digestion by the method of Olson *et al.* (7). Vitamin A and carotene determinations on the unsaponified residue were made according to the method of Koehn and Sherman (6).

### RESULTS

The daily feeding of 60 cc. of shark liver oil with a Vitamin A potency of 25,000 I.U. per gram had no effect on milk or butterfat production. These data are presented in figure 1 and table 1.

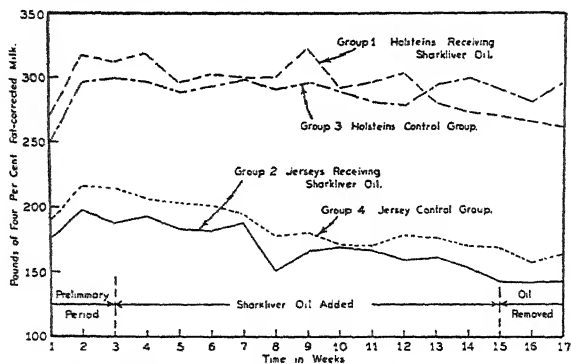


Figure 1. Average pounds of four per cent fat-corrected milk produced per week.

FIG. 1. Average pounds of four per cent fat-corrected milk produced per week.

The data in table 1 show that the feeding of shark liver oil caused a marked increase in the Vitamin A content of the butterfat. This increase was approximately 280 per cent for the Holstein cows and 305 per cent for the Jersey cows. The vitamin A content of the butterfat reached its greatest value the second week after the cows received the supplement. After this the Vitamin A content of the butterfat dropped somewhat, but was maintained at a considerably higher level than that of the cows receiving no supplement.

The carotene content of the butterfat of the cows receiving the supplement gradually decreased during the course of the experiment (table 1).



TABLE 1

*The effect of shark liver oil feeding on the milk and fat production and the vitamin A and carotene content of the milk fat of Holstein and Jersey cows*

Period week ending	Group I Holstein Basal ration + 60 cc. shark liver oil			Group III Holstein Basal ration		
	Av. weekly milk yield, lbs. 4% F.C.M.	Av. vita- min A, I.U./gm. milk fat	Av. caro- tene, micro- grams/gm. milk fat	Av. weekly milk yield, lbs. 4% F.C.M.	Av. vita- min A, I.U./gm. milk fat	Av. caro- tene, micro- grams/gm. milk fat
Preliminary period						
1- 9-42	269.7	.....	.....	248.1	.....	.....
1-16-42	318.7	24.2	4.4	297.0	33.2	3.9
1-23-42	312.6	18.6	3.6	299.8	16.9	2.6
1-30-42	319.6	20.3	3.0	293.6	19.8	2.4
Experimental period						
2- 6-42	297.6	52.6	3.2	289.0	20.5	2.0
2-13-42	303.2	69.0	3.6	293.3	23.7	2.9
2-20-42	301.5	44.3	3.0	299.5	22.0	2.9
2-27-42	301.4	37.7	2.9	292.4	20.7	2.8
3- 6-42	291.6	54.4	2.9	297.8	20.5	2.8
3-13-42	293.9	66.5	2.2	290.2	21.3	2.4
3-20-42	287.7	64.9	3.2	282.0	20.1	2.7
3-27-42	304.7	53.0	2.4	279.3	20.3	2.9
4- 3-42	280.7	57.9	2.4	294.6	20.9	2.5
4-10-42	274.0	45.4	1.8	299.8	16.3	3.4
4-17-42	271.4	52.8	1.9	296.3	15.7	2.4
4-24-42*	266.2	55.0	2.7	280.5	21.2	3.6
5- 1-42	262.3	42.1	3.6	297.2	31.0	4.3
	Group II Jersey			Group IV Jersey		
Preliminary period						
1- 9-42	175.5	.....	.....	190.0	.....	.....
1-16-42	198.3	18.7	6.2	216.7	20.5	6.0
1-23-42	187.5	15.3	5.3	214.0	16.6	4.1
1-30-42	193.1	12.4	4.2	206.3	12.6	3.3
Experimental period						
2- 6-42	184.2	43.7	4.5	204.1	24.2	3.9
2-13-42	181.7	71.5	4.5	202.9	29.3	4.3
2-20-42	225.1	39.2	3.9	195.7	18.6	4.2
2-27-42	181.3	48.1	3.9	177.9	23.5	4.5
3- 6-42	166.2	44.3	3.2	180.7	23.8	3.9
3-13-42	168.8	53.4	3.0	171.2	24.7	3.6
3-20-42	166.1	49.3	3.6	170.9	24.9	3.7
3-27-42	159.2	39.4	2.8	178.1	28.9	3.8
4- 3-42	161.8	53.6	3.5	176.9	25.2	4.3
4-10-42	153.2	39.8	2.9	170.2	22.8	4.3
4-17-42	142.9	42.3	3.1	165.9	22.9	3.8
4-24-42*	141.8	51.4	2.9	190.6	24.7	4.9
5- 1-42	142.1	29.8	4.6	164.8	25.9	6.1

\* Shark liver oil removed from ration.

There was an average reduction of 22 per cent in carotene content of the Holstein butterfat, and 33 per cent in the carotene content of the Jersey butterfat.

#### DISCUSSION

The data presented show that the daily administration of 60 cc. of shark liver oil furnishing a daily intake of 1,350,000 I.U. of Vitamin A had no effect on milk or butterfat production. This supports the results of Jensen *et al.* (5) and is contrary to the findings of Deuel and co-workers (1, 4).

The increase in the Vitamin A content of the milk is in agreement with the results of Deuel and co-workers (2, 4) and Jensen *et al.* (5).

While the increase in Vitamin A content of milk fat was not as marked as that reported by Deuel, it was significant. The decrease in carotene content of the butterfat supports the results of Deuel (3) and of Jensen *et al.* (5).

#### SUMMARY

1. The daily feeding of 60 cc. of shark liver oil furnishing 1,350,000 I.U. of Vitamin A had no effect on milk or butterfat production of either Holstein-Friesian or Jersey cows.

2. The daily feeding of 60 cc. of shark liver oil resulted in a 280 to 305 per cent increase in the Vitamin A content of the milk fat.

3. The feeding of shark liver oil caused a 22 per cent average decrease in the carotene content of the Holstein milk fat, and a 33 per cent average decrease in the carotene content of the Jersey milk.

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# A 2×2 FACTORIAL DESIGN FOR DOUBLE REVERSAL FEEDING EXPERIMENTS

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Investigators in the dairy field have for years made liberal use of the double-reversal design for conducting feeding trials. This design employs the procedure of using two groups of cows (A and B) and starting period I with the A group on ration X and the B group on ration Y. At the end of each period the rations are reversed; thus, in period III the groups are fed the same rations as in period I. In the analysis the production during period II is compared to the average for periods I and III. The comparison thus made is considered appropriate because of the usual decline in production of cows as the lactation progresses. The assumption is made that if other factors remain under control the cows will produce as much in period II as the average of periods I and III.

One of the strongest criticisms against employing the double reversal design is that there is a residual, or carry-over effect, caused by the previous rations fed. In 1941 Cochran, Autrey and Cannon (1), in a discussion of a double change-over design presented a method for correcting this influence. A partial correction for this carry-over effect is also made possible by discarding the records for a preliminary period at the beginning of each feeding period.

A method for determining the significance of the differences found in the reversal or switch-back trials was presented by Brandt in 1938 (2). An extension of this test is herein presented so as to permit the experimenter to compare two sets of factors at the same time—such as two concentrates and two roughages—with an appropriate method of testing the significance of the differences found between each set of factors. The design where applicable can employ the same number of cows to answer two questions as is commonly used to answer a single question. Caution should be exercised in its use, however. The experimenter should know from previous knowledge or experience that the differences between the two roughages is likely to be of about the same magnitude while on each of the two concentrate mixtures.

## THE EXPERIMENTAL PLAN

The particular design presented grew out of an attempt to use a limited number of cows in two feeding trials to compare two kinds of hay and two concentrate mixtures. Rather than conduct separate trials it was decided

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to combine the trials with the major group, A or B, getting the same kind of hay (alyce or lespedeza)<sup>2</sup> and the subgroups, a and b, differing in the kind of concentrate, either three parts corn and one part cottonseed meal or three parts dehydrated sweet potato meal and one part cottonseed meal. Twenty

TABLE 1

*Alyce clover vs. lespedeza and corn vs. dehydrated sweet potato meal in the dairy ration  
(Production of 4% equivalent milk)*

Hay group	Grain group	Cow No.	Period I	Period II	Period III	$P_1 - 2P_2 + P_3$
			$P_1$	$P_2$	$P_3$	
A			Alyce + s. pot.	Lesp. + corn	Alyce + s. pot.	
	a	1	369.7	337.6	303.9	- 1.6
		2	376.1	380.8	413.8	+ 28.3
		3	440.0	376.0	407.4	+ 95.4
		4	375.0	362.2	344.5	- 4.9
		5	248.5	257.5	278.5	+ 12.0
		Sums	1809.3	1714.1	1748.1	+ 129.2
			Alyce + corn	Lesp. + s. pot.	Alyce + corn	
	b	1	206.4	196.1	210.6	+ 24.8
		2	486.0	423.4	469.7	+ 108.9
		3	425.0	350.7	373.6	+ 97.2
		4	342.5	302.9	362.9	+ 99.6
		5	474.9	422.5	442.3	+ 72.2
		Sums	1934.8	1695.6	1859.1	+ 402.7
B			Lesp. + s. pot.	Alyce + corn	Lesp. + s. pot.	
	a	1	182.1	207.7	199.7	- 33.6
		2	436.1	501.0	430.5	- 135.4
		3	341.3	378.0	338.18	- 76.6
		4	373.9	427.8	374.3	- 107.4
		5	383.1	404.6	345.8	- 80.3
		Sums	1716.5	1919.1	1688.4	- 433.3
			Lesp. + corn	Alyce + s. pot.	Lesp. + corn	
	b	1	257.1	253.3	239.5	- 10.0
		2	436.7	461.3	487.9	+ 2.0
		3	489.3	484.3	441.0	- 38.3
		4	308.4	332.3	307.8	- 48.4
		5	235.5	245.0	248.6	- 5.9
		Sums	1727.0	1776.2	1724.8	- 100.6

cows were used in the trial with 10 in each hay group and these were subdivided into two five-cow concentrate groups. Table 1 presents the plan

<sup>2</sup> The lespedeza hay used was approximately one grade lower in quality than was the alyce clover hay. The comparisons made are for the purposes of presenting a method of analysis rather than to give experimental results.

along with the resulting milk yields for the cows for each of the three periods. Each period consisted of 21 days: 7 days as a preliminary period and 14 days as a test period.

#### THE ANALYSIS

##### 1. *An Example Showing Significant Differences*

The present experiment conforms to a factorial pattern, an experimental design described in detail by Yates (4). This particular factorial design involves variations in two sets of factors, so is designated as a  $2 \times 2$  factorial, and has the interaction between the two sets of factors confounded with cows. Our notation on the factors involved is:

<i>First factor</i>	<i>Second factor</i>
1 Alyce clover	1 Sweet potato
2 Lespedeza	2 Corn

Using this notation the sub-group totals in table 1 are:

$$A(a) \ 11 - 22 = +129.2$$

$$A(b) \ 12 - 21 = +402.7$$

$$B(a) \ 21 - 12 = -433.3$$

$$B(b) \ 22 - 11 = -100.6$$

The sub-total differences are derived by adding sums for Periods I and III and subtracting twice the sum for Period II. Thus, 11 - 22, or the sub-group difference in production between alyce + sweet potatoes and lespedeza + corn equals  $1809.3 - 2(1714.1) + 1748.1$  or +129.2. A study of the subgroup totals immediately gives evidence that alyce clover + corn was the best combination, for this ration as shown in B(a) excelled lespedeza and sweet potatoes by 433.3 pounds of milk. This type of comparison, however, involves possible first-order interactions which are confounded with cows in this experiment. These interactions are ignored and the first problem at hand is to determine the differences between the two kinds of hay and the two concentrate mixtures. They are:

$$\text{alyce} - \text{lespedeza} = 129.2 + 402.7 - (-433.3) - (-100.6) \text{ or } 1065.8$$

$$\text{S. pot.} - \text{corn} = 129.2 + (-433.3) - 402.7 - (-100.6) \text{ or } -606.2$$

These differences show in one case the superiority of alyce clover over lespedeza and in the other the inferiority of sweet potato meal as compared to corn. The next procedure is to determine the significance of these differences.

The Analysis of Variance employing the calculation of the sum of squares for a single degree of freedom as described by Snedecor (3), will first be used. In terms of a single difference ( $P_1 - 2P_2 + P_3$ ) the sum of squares for these components are:

$$\frac{(1065.8)^2}{20^*} = 56,796$$

$$\frac{(-606.2)^2}{20} = 18,373$$

\* The denominator equals  $nk$  or the number of major groups (2)  $\times$  the number of cows in each group (10) or 20. See (3).

The individual cow differences as well as the sub-group totals under the heading  $P_1 - 2P_2 - P_3$  in table 1 are employed in computing the error sum of squares, thus:  $(-1.6)^2 + (28.3)^2 + (95.4)^2 \dots + (5.9)^2 - 1/5 [(129.2)^2 + (402.7)^2 - (-433.3)^2 + (-100.6)^2] = 26,703$  with 16 degrees of freedom. This gives for the analysis of variance:

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Alyce vs. lespedeza .....	1	56,796	56,796	34**
S. pot. vs. corn .....	1	18,373	18,373	11**
Error .....	16†	26,703	1,669	.....

The analysis shows that both sets of factors were significantly different at the one per cent level\*\*; with alyce clover excelling lespedeza, and corn excelling sweet potato meal.

An analysis involving the use of group means and testing the significance by the use of the standard error will next be presented. Table 2 shows a  $2 \times 2$  table with totals. The treatment means per cow per period with differences, and the respective standard errors of these differences are also given.

TABLE 2  
Milk yields by treatments—with means and differences

	Alyce	Lepedeza	Mean	Difference
S. potatoes .....	355.5	339.8	347.6	15.2 ± 4.567
Corn .....	381.6	344.0	362.8	
Mean .....	368.6	341.9		
Difference .....	26.7 ± 4.567			

Calculating these means on a per cow per period basis involves using sub-group totals from table 1; thus, for alyce + s. pot. =  $1809.3 + (2 \times 1776.2) + 1748.1/20$  or 355.5. In like manner the other three totals in table 2 are calculated. From this the variance of a figure in this  $2 \times 2$  table becomes:

$$5/400 [1^2 + 2^2 + 1^2] \times (\text{variance per cow per period}) =$$

$$3/40 \sigma^2, \text{ where } \sigma^2 = \frac{1,669\dagger}{6}. \text{ This gives } 20.86.$$

The standard error of the difference =  $\sqrt{20.86}$  or 4.567

$$t = \frac{\text{Mean difference}}{\text{S.E.}} = \frac{26.7}{4.567} = 5.85^{**}$$

Likewise

$$\frac{15.2}{4.567} = 3.33^{**}$$

The values of  $t$ , as was the case with  $F$  in the analysis of variance, are significant at the one per cent point. The  $t$  and  $F$  tests are, of course, identical.

† The error term has 16 degrees of freedom, four from each group.

‡ The method for obtaining 1669 is given for Error Variance in the analysis of variance and 6 is the sum of exponents  $1^2 + 2^2 + 1^2$ .

## 2. An Example Showing non-Significant Differences

Liveweight changes as well as milk and butterfat yields are used as "measuring sticks" in double reversal feeding trials. Their use in the experiment just described furnishes data to illustrate a case where the differences observed failed to be significant at the five per cent level. The use of the analysis of variance to test this significance will be presented.

Table 3 presents the liveweight changes for each cow and the sums for the sub-groups. The effects of the rations are shown by

$$\text{Alyce-lespedeza} = (-64 - 434) - (-33) - (-118) \text{ or } -413$$

$$\text{S. pot.-corn} = (-64 - 33) - (-434) - (-118) \text{ or } 521$$

TABLE 3  
Summary of liveweight changes

Hay group	Grain group	Cow No.	Period I P <sub>1</sub>	Period II P <sub>2</sub>	Period III P <sub>3</sub>	P <sub>1</sub> - 2P <sub>2</sub> + P <sub>3</sub>
A	a		Alyce + s. pot.	Lesp. + corn	Alyce + s. pot.	
		1	+ 38	- 18	- 25	- 23
		2	- 10	- 15	+ 42	+ 82
		3	- 3	+ 45	- 10	- 103
		4	+ 9	+ 10	- 12	- 23
		5	- 14	- 8	+ 33	+ 3
		Sums	+ 40	+ 66	+ 28	- 64
	b		Alyce + corn	Lesp. + s. pot.	Alyce + corn	
		1	- 7	+ 29	- 5	- 70
		2	- 16	+ 12	+ 4	- 36
		3	- 64	+ 79	- 15	- 237
		4	+ 22	+ 13	- 1	- 5
		5	+ 1	+ 37	- 13	- 86
B	a		Lesp. + s. pot.	Alyce + corn	Lesp. + s. pot.	
		1	+ 13	- 29	+ 9	+ 80
		2	+ 20	+ 2	+ 5	+ 21
		3	- 25	+ 23	+ 0	- 71
		4	- 12	- 9	+ 22	+ 52
		5	- 13	+ 28	- 6	- 49
		Sums	+ 33	+ 15	+ 30	+ 33
	b		Lesp. + corn	Alyce + s. pot.	Lesp. + corn	
		1	- 1	+ 14	- 18	- 47
		2	+ 24	+ 9	- 10	- 4
		3	+ 27	+ 35	+ 19	- 24
		4	+ 4	+ 3	+ 22	+ 20
		5	+ 9	+ 38	+ 4	- 63
		Sums	+ 63	+ 99	+ 17	- 118

It appears from these differences that lespedeza excelled alyce hay and that sweet potato meal excelled corn in maintaining liveweight.

In terms of single differences ( $P_1 - 2P_2 - P_3$ ) the sum of squares for the two sets of factors becomes:

$$\frac{(-413)^2}{20} = 8,528$$

$$\frac{(521)^2}{20} = 13,572$$

The error sum of squares is  $(-23)^2 + (52)^2 + \dots + (-63)^2 - 1/5 (-64)^2 + (-434)^2 + (33)^2 - (-118)^2 = 70,850$

<i>Source of variance</i>	<i>Degrees of freedom</i>	<i>Sum of squares</i>	<i>Mean square</i>	<i>F</i>
Alyce vs. lesp. ....	1	8,528	8,528	1.9
S. pot. vs. corn ....	1	13,572	13,572	3.1
Error .....	16	70,850	4,428	....

In this test neither sets of factors are significantly different at the five per cent level. The size of the F value for sweet potato meal vs. corn might lead the experimenter to be suspicious that a real difference did exist and cause him to repeat the experiment in an effort to determine the accuracy of the first information secured.

#### SUMMARY AND CONCLUSIONS

An efficient method of using the double reversal experimental design to answer two questions with a limited number of cows is presented. The design makes it possible to compare two roughages and also two concentrate mixtures in the same feeding trial. The appropriate use of this design is where previous knowledge or experience indicates that the first degree interaction is unimportant, *i.e.*, that differences between the concentrate mixtures will be approximately the same while on each roughage.

Appropriate tests for measuring the differences based on a  $2 \times 2$  factorial design are presented. These tests utilize the analysis of variance or the t-test with identical results. Examples given include milk yields showing significant differences between both roughages and concentrates, and liveweight changes, with non-significant differences between each set of factors.

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ANNOUNCEMENT  
THIRTY-NINTH ANNUAL MEETING  
OF  
THE AMERICAN DAIRY SCIENCE ASSOCIATION  
COLUMBUS, OHIO, JUNE 20-22, 1944

NOTE REGARDING THE PROGRAM

In planning the program for the Columbus meeting of the American Dairy Science Association, the officers and members of the Program Committee are endeavoring to focus the program upon the problems of America at War and the problems following, during the peace to come. In order to formulate a program of the most usefulness and interest, it is essential that the committee receive the titles of proposed papers at as early a date as possible, but certainly not later than April 1. Titles will be carefully considered by the committee and abstracts of the papers, which, unless otherwise stated, are assumed to be not longer than 12 minutes, must be in the hands of the Chairman, Professor H. P. Davis, Dairy Husbandry Department, University of Nebraska, Lincoln 1, Nebraska, not later than June 1.

TENTATIVE PROGRAM OUTLINE

TUESDAY, JUNE 20, 1944

*Morning Session*

Welcome by representative of the host institution.  
Response and address by President Dahlberg.  
Address by guest speaker.

*Afternoon Session*

Sectional meetings.  
4:00 P.M. Section business meetings.

WEDNESDAY, JUNE 21, 1944

*Morning Session*

Section meetings.  
11:00 A.M. Section business meetings.

*Afternoon Session*

General session on post war problems.

THURSDAY, JUNE 22, 1944

*Morning Session*

Joint symposium—Production and Extension Sections.  
Manufacturing symposium.

*Afternoon Session*

General session on Latin American Dairying.

3:30 P.M. Association business meeting.

*Evening Session*

Annual Banquet.

H. P. DAVIS

*Chairman, Program Committee*

## NEW PROCEDURE TO NOMINATE CANDIDATES FOR OFFICE

In accordance with the provisions of the revised constitution adopted last year, your president has appointed a committee on nominations whose names appeared in the January issue of the Journal and are again listed below. It is the duty of this committee of which J. H. Frandsen is chairman to send its report to the secretary not later than April 1. The secretary will then mail out the ballots to secure a final vote before the annual meeting. The results of the election will be announced at the annual meeting.

As a member of the Association, it is your obligation to express your wishes for officers and directors to any member of this committee. Two candidates for vice-president and four for directors will be nominated. The present vice-president automatically becomes president. The committee on nominations will give consideration to the wishes of the members as expressed by correspondence and will also consider additional candidates. They have been advised by your president to study the list of past officers and directors (see pages 803-804 of the August Journal) to plan to secure good geographic distribution and to recognize the desirability of representation from all lines of activity of our members. This information has been given in detail to the committee for their guidance.

It was the intention of our Association to make the election of officers just as democratic as possible, and this can be done only if members do promptly give their opinions freely to the committee on nominations.

A. C. DAHLBERG, *President*

## COMMITTEE ON NOMINATIONS

J. H. FRANDSEN, *Chairman*

H. A. RUEHE

J. F. KENDRICK

C. N. SHEPARDSON

H. P. DAVIS

# JOURNAL OF DAIRY SCIENCE

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## RELATIONSHIP OF FAT ACIDITY TO RANCIDITY IN HOMOGENIZED RAW MILK\*

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The action of milk lipase on butter fat is generally believed to produce at least two detectable changes: first, the production of free fatty acids, and second, the production of rancid or rancid-like flavors. The production of the rancid flavor is attributed to free butyric acid and other lower fatty acids such as capric, caprylic, and caproic. Furthermore, the appearance of the rancid flavor is expected whenever milk fat undergoes lipolysis, indicating that the glycerides of the lower fatty acids are always attacked by lipase under normal conditions. Some efforts have been made to correlate the extent of fat splitting with rancid flavor development (1, 5, 7), but information on this relationship is by no means complete nor has it been applied to conditions in which the lipase activity is accelerated by homogenization.

The amount of lower fatty acids liberated from butterfat by lipase action may be expected to be relatively small inasmuch as the glycerides of the lower fatty acids constitute a comparatively small portion of the total fat and, also, since the work of Willstatter and Memmen (10) indicates pancreatic lipase to have slight affinity for the lower esters.

Although the quantity of the lower fatty acids involved in lipase activity is small, the effect of these acids on flavor may be marked. Grossfeld and Battay (3) observed butyric acid could be detected by smell when present in a ratio of 1:12,500. In the case of dairy products, the actual type of flavor produced by lipase activity may not always be well defined and may vary with conditions. Relatively slight lipase activity may result in flavor defects which are not typically rancid (4, 7).

Results which have been reported showing the relationship between the acid degree of the fat (ml. of N NaOH per 100 grams of fat) and the rancid flavor in the original product from which the fat was obtained are not in total agreement. Fouts (1) in his study of commercial butter, could find no direct relationship between rancidity of the butter and the acid degree of the butterfat. The acid degrees of fat from non-rancid butter ranged

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from 2.9 to 11.6, whereas values for rancid butter ranged from 2.4 to 14.0. In those samples which were rancid, there was no relationship between intensity of rancidity and free fatty acid content. Recently, Krukovsky and Herrington (6) and Jack, Tarassuk, and Scaramella (5) conducted controlled experiments in which butter was manufactured from milk having naturally active lipase. Krukovsky and Herrington found the rancid flavor to appear in butter between acid degrees of 0.75 and 2.0 with the lower value being near the flavor threshold of rancidity. Jack *et al.* concluded rancidity of butter was recognized organoleptically when the acid degree approached a value of 2.0 and that direct correlation exists between acid degree and rancid flavor.

In experiments with milk, Krukovsky and Herrington (7) determined "the acid degree at which rancidity could first be recognized as such." Although some variations occurred, the threshold at which rancidity was recognized in the milk was approximately at an acid degree of 0.8. However, the data indicated a tendency for the judges to lower the flavor score of milk as a result of lipase action even before the nature of the defect was recognized. Samples with an acid degree above 1.4 were graded in the low-fair and poor class.

The studies of Krukovsky and Herrington (7) were concerned with normal milk and do not necessarily apply to homogenized milk in which lipolysis is accelerated. This is indicated by the results of Gould and Trout (2) which showed homogenization to result immediately in greatly increased acid degrees in the fat, and also, by the observations of Trout and Scheid (9) that certain commercial plants homogenized raw milk and then pasteurized it immediately without any detectable flavor change. Trout and Scheid also demonstrated the feasibility of this practice. Lane and Hammer (8) observed the flavor of cheese made from a mixture of raw skim milk and raw homogenized cream to be good even though the fat acidity was high (acid degree 9.7 in one case, 8.2 in another). However, flavor production in cheese is not necessarily analogous to that of milk.

The experiments herein reported were conducted with the view of securing information on the relationship between the acidity of fat from homogenized raw milk and the rancid flavor. The problem was investigated from two angles: (a) by homogenizing raw milk and then making fat and flavor examinations at different intervals, (b) by homogenizing fat possessing different acid degrees and secured from non-homogenized and homogenized raw milk into pasteurized skim milk and then examining the mixture for the rancid flavor.

#### EXPERIMENTAL PROCEDURE

Milk used in these studies was selected from milk delivered to the College Creamery, care being used to obtain milk practically free of off-flavors. Homogenization of this milk was at 700 pounds pressure and at 37° C. Fat

was secured for analysis by separation, churning and filtering. Ten gram samples were titrated for free fatty acids with 0.05 N NaOH. The free fatty acid content is expressed as acid degree (mls. of N NaOH per 100 gms. of fat).

#### EXPERIMENTAL RESULTS

*Homogenized raw milk.* In this phase of the study, selected raw milk was standardized to approximately 10 per cent fat by separation and this milk then divided into two portions. One portion served as the control and was non-homogenized, the other portion was homogenized at 700 pounds pressure and then stored at approximately 25° C. A sample of the homogenized milk was secured immediately after homogenization and at intervals thereafter (usually 15-minute intervals) until the milk had become definitely rancid. At specified intervals, samples, including the control, were

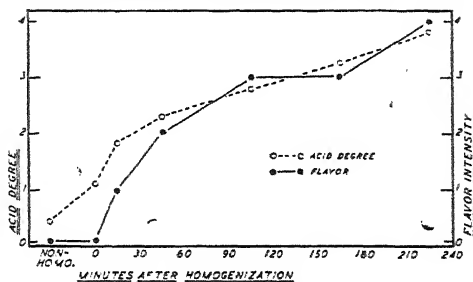


FIG. 1. Relationship between intensity of rancid flavor and increases in acid degree following homogenization of the milk at 700 pounds pressure.

quickly heated to 142–144° F. for 20 minutes and then cooled. After samples for the trial were obtained, portions of each were examined “blind” organoleptically, and other portions were used to supply the fat for titration. Seventeen trials were conducted and six to nine samples were examined for changes in flavor and fat acidity in each trial.

Average results of four trials in which the samples were secured at the same intervals before and following homogenization are illustrated in figure 1. This figure portrays (a) the general relationship between increase in acid degree and development of rancidity; (b) the fact that considerable increase in acid degree may occur before the rancid flavor is detectable but that rancidity develops within a few minutes after homogenization.

Homogenization increased the acid degree of the fat approximately three-fold, i.e., from 0.43 to 1.2, without producing a rancid flavor that was detectable by taste. Within 15 minutes the acid degree had increased to approximately 1.8 and the judges usually graded the flavor as “questionable” in regard to the rancid flavor. By the end of 30-minute and 45-minute periods the samples were usually rancid and the acid degree of the fat for the 45-minute period averaged approximately 2.3.

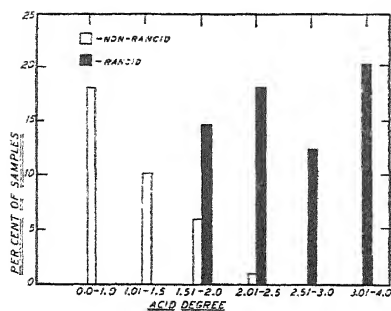


FIG. 2. Distribution of non-rancid and rancid samples of homogenized milk on basis of acid degree of the fat.

The relationship between acid degree and rancidity of all of the samples examined in the 17 trials is illustrated by figure 2. This graph reveals that the rancid flavor was generally detectable when the acid degree was within the range of 1.5 to 2.0. Below an acid degree of 1.5 no samples were criticized as being rancid whereas practically the reverse condition held when the acid degree was above 2.0. Of all the samples examined, approximately one-third were adjudged "non-rancid," with 28 per cent having acid values of 1.5 or less. Only one sample having an acid degree above 2.0 was graded non-rancid.

*Fat from non-homogenized and homogenized raw milk:* In the second phase of this portion of the study, fat of varying acidities was secured from non-homogenized and homogenized milk. This fat was then re-homogenized into pasteurized skimmilk in proportions to give a 5 per cent milk and the mixture examined organoleptically. Results of several typical trials are presented in table 1.

These data reveal that in no case was a rancid flavor produced in the fat-skimmilk mixture even though the acid degree of the fat ranged from 0.5 to 11.5. Instead, an oxidized, oily, or tallowy flavor resulted in the samples; this flavor appearing in low acid-degree samples as well as in the high, but to

TABLE 1

*Effect on the flavor of the mixture when butter fat of varying acid degrees is homogenized into skim milk*

	Trial 1		Trial 2		Trial 3	
	Acid degree of fat	Flavor of mixture	Acid degree of fat	Flavor of mixture	Acid degree of fat	Flavor of mixture
Skimmilk .....	.....	OK	.....	OK	.....	OK
Skimmilk plus fat .....	0.5	Oxidized	0.5	Oxidized	0.5	Oxidized
Skimmilk plus fat .....	1.5	Oxidized	1.5	Oxidized	6.5	Oxidized
Skimmilk plus fat .....	2.5	Oxidized	2.5	Oxidized	0.6	Oxidized
Skimmilk plus fat .....	3.5	Oxidized	4.0	Oxidized	7.5	Oxidized
Skimmilk plus fat .....	4.3	Oxidized	.....	.....	0.5	Oxidized
Skimmilk plus fat .....	5.6	Oxidized	.....	.....	11.5	Oxidized

slightly less intensity. The degree of oxidized flavor was never sufficient to have obscured the rancid flavor.

Examination of the butteroil used in these trials for the rancid flavor also gave negative results, the oil possessing an oxidized flavor when the acid degree was high. However, when the acid degree was less than 1.0, the butteroil did not uniformly exhibit the oxidized flavor. Although the fat itself, following the removal of the curd and water by filtration, did not possess the rancid flavor, the buttermilk obtained by the churning of cream with a high acid degree was always intensely rancid.

#### DISCUSSION

The direct relationship between the development of rancidity in milk and the increase in acid degree of the fat as observed in this experiment is in agreement with the findings of Krukovsky and Herrington (7). However, the threshold value for the flavor is approximately twice as high in these studies on homogenized milk as was reported by Krukovsky and Herrington for normal milk. This difference may be explainable on the basis that when lipolysis is accelerated by homogenization, lipase activity proceeds at the expense of those fatty acids which contribute to the titratable acidity of the fat at a proportionately greater rate than is true in the case of non-homogenized milk.

The results secured in this study serve as a basis for explaining the differences which have been reported relative to the correlation between the acid degree of the fat and the rancid flavor. The lack of such a correlation in commercial butter observed by Fouts (1) is understandable in view of the fact that the rancid flavoring materials in the milk or cream are largely lost in the buttermilk when the fat is obtained by churning, even though the fat itself shows the presence of appreciable quantities of free fatty acids. Whether or not butter from rancid cream will retain the rancid flavor would appear to be dependent upon the amount of buttermilk incorporated into the butter, and thorough washing of the butter may completely wash away the rancid-flavored fatty acids. The author has observed that the churning of rancid cream did not uniformly result in rancid butter. This does not eliminate the possibility, however, of the butter becoming rancid on storage due to continued lipase activity. The problem of water solubility of the fatty acids responsible for the rancid flavor is considered more thoroughly in the subsequent paper.

The fact that samples of butteroil with high acid degree produced an oxidized or oily flavor when homogenized in skimmilk does not necessarily mean that there is a relationship between free fatty acid content and the oxidized flavor under these conditions. Such a relationship was not apparent since fat of low acid degree also produced this flavor in the milk. The important point is that the rancid flavor was not produced by this process, even though the fat was obtained from rancid homogenized raw milk.

The apparent absence of at least a portion of the lower fatty acids from butteroil prepared by churning the cream and filtering the fat as indicated by the absence of the rancid flavor may tend to cast some doubt as to the reliability of the titration of the fat as a means of measuring lipolysis. It does appear that such titration values do not necessarily represent all of the fatty acids that are freed by lipase activity, although the results of Gould and Trout (2) would indicate, at least, almost complete measurement of these acids. To secure greater accuracy it may be desirable to utilize some other method than churning in obtaining the fat from the milk or cream. The author has made use of an extraction procedure and the results secured were encouraging. Higher fat titers were secured in the extracted fat than in the churned fat, but the possibility that these higher values may be due to the inclusion of other than fatty acids has not been eliminated.

#### CONCLUSIONS

Raw milk, homogenized at 700 pounds pressure, is usually rancid when the acid degree of the fat is within the range of 1.5 or 2.0.

Fat secured from rancid milk and with acid degrees as high as 11.5 did not itself possess a rancid flavor nor did it produce a rancid flavor when homogenized into pasteurized skim milk.

Free fatty acids in butterfat which is obtained by churning are not responsible for the typical rancid flavor of dairy products.

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## SOLUBILITY AND VOLATILITY OF FATTY ACIDS INVOLVED IN LIPOLYSIS IN HOMOGENIZED RAW MILK\*

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Homogenization of raw milk, a process known to greatly accelerate lipase activity, results in immediate increase in the free fatty acid content of the fat with homogenization at 1500 pounds pressure increasing the free fatty acids approximately five-fold within a few minutes following processing (6). Since information relative to the characteristics of the fatty acids involved in this lipolysis is meager, experiments were conducted to ascertain their solubility and volatility. The results of these experiments are herein presented.

Steam distillation procedures have been used to study the volatility of free fatty acids in milk and milk products. Roahen and Sommer (9) used this procedure to measure the extent of lipolysis in milk and cream and Fouts (4) studied the volatility of free fatty acids in butteroil of varying acid degrees secured from butter by steam distillation of 10 grams of fat and collecting and titrating 200 ml. of distillate. He secured values for volatile fatty acids approximating 15 per cent of the total fat acidity. Davies (2) reports that volatile acids account for less than five per cent of the total titratable value of the fatty acids, whereas oleic acid is responsible for 60-70 per cent of this value.

Dyer (3), and Hiscox and Harrison (7), used steam distillation in the study of pure volatile organic acids. Hiscox and Harrison (7) steam distilled acetic, butyric, caproic, caprylic, capric, and lauric acids (acids neutralized with NaOH and then acidified to pH 2 with  $H_2SO_4$ ) and secured complete recovery from water when distillate volume was "5 times original volume." Recovery of these acids from cheese was retarded, the retardation being especially marked for acetic, caprylic, caproic, and lauric acids. Butterfat and cheese fat also retarded distillation of the water-insoluble fat-soluble acids.

Methods of preparing samples of cheese for steam distillation were studied by Hiscox, Harrison, and Wolf (8). They found that a method involving warm water washing of the cheese to obtain the fat, ether extraction of the fat, washing of the fat with NaOH, acidifying and steam-distilling this rinse, resulted in volatile acid values which were about 4.5 times as high as those from direct steam distillation in the case of Stilton cheese and about twice as high in the case of Cheddar.

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## PROCEDURE

The studies reported in this paper were conducted with two purposes in mind: (a) to determine the solubility of fatty acids in fat from homogenized raw milk by washing the fat either with water or with a weak alkaline solution; (b) to determine the volatile fatty acid content of the butterfat by direct steam distillation of the butterfat itself and by the distillation of the alkaline rinsings of the butterfat.

Butterfat with high free fatty acid content was produced by homogenization of raw milk at 37° C. and at 500 pounds pressure. The butterfat was secured by churning, melting, centrifuging, and filtering.

Fat acidity was determined by the official procedure (1) with exception that a 10-gram sample, 25 ml. ethyl alcohol, and N/20 NaOH solutions were used. The free fatty acid content is expressed as acid degree (mls. of N NaOH per 100 gms. of fat).

A multiple steam distillation apparatus, capable of distilling four samples at once, was utilized for the studies on volatile acids. This apparatus consisted of a sodium chloride bath maintained at a temperature of 102–104° C. The bath was of sufficient size to accommodate four one-liter round bottom flasks containing the samples. Steam was generated in two five-liter round bottom flasks, each flask supplying steam for two samples. The steam entered the sample through Folin-type ammonia tubes with perforated bulbs at the bottom, and this inlet tube extended well below the surface of the samples. A Kjeldahl connecting bulb was placed at the outlet from the flask to insure against entrainment of the sample. Condensation of the steam enroute to and from the sample flask was reduced to a minimum by wrapping the conducting glass tubes with asbestos. By regulation of the temperature of the bath and the rate of steam it was possible to maintain fairly constant volume in the flasks containing the samples.

The volatile material leaving each sample passed to a Liebig condenser where it was condensed. The distillate then passed through a No. 40 Whatman filter paper to remove the water insoluble acids and into a 200-ml. volumetric flask. Titrations were conducted in each 200 ml. sample with 0.05 N NaOH, using phenolphthalein as the indicator. After each 200 mls. of distillate were secured, the condensers and the filters were washed with ethyl alcohol to dissolve the alcohol-soluble fatty acids. This alcohol fraction was titrated and the titer added to the values secured for the 200 mls. of distillate.

Blank distillations were made on water at pH 2. The blank values, which included those for the alcohol used to rinse down the condensers, averaged approximately 0.35 ml. of 0.05 N NaOH per 200 mls. of distillate.

## RESULTS

*Solubility of free fatty acids.* In other work (5) the senior author observed that butterfat, secured from rancid cream by churning and filtering,

and with a high free fatty acid content did not possess a rancid flavor. This observation raised the question as to the water solubility of these fatty acids and trials were conducted to ascertain their solubility by washing the butterfat with warm water. In this experiment, approximately 20 gms. of butterfat having a high content of free fatty acids (acid degree approximately 5) were placed in a separatory funnel and shaken for 30 seconds with 20 ml. of water at 130–135° F. The fat layer was then permitted to rise and the aqueous layer removed and titrated. This washing was repeated six times. After the final washing, the fat was removed, dried, and titrated. Results of the original and final titrations on the fat are shown in table 1.

These results show no decrease in the acid degree of the fat to occur with six washings with warm water. Although the data are not shown, titrations of the washings revealed no measurable quantity of fatty acids were contained therein. These findings indicate either that the water-soluble fatty acids are not retained by the fat during its churning and purification or that they have greater affinity for the fat than for the water.

TABLE 1  
*Influence of washing butterfat with water on the acid degree*

	Trial 1	Trial 2	Trial 3	Trial 4
Original acid degree of fat .....	4.93	4.87	4.82	4.80
Final acid* degree of fat .....	4.91	4.88	4.85	4.85

\* Following washing of 20 grams of fat with six successive portions of warm water.

Other trials were conducted to determine the efficiency with which a weak alkaline solution may remove the fatty acids from fat. The method used was similar to that proposed by Hiscox *et al.* (6) for cheese. Fat samples weighing either 25 or 50 grams were dissolved in 100–120 mls. ethyl ether in a separatory funnel. Twenty-ml. portions of 0.05 N NaOH were added and the mixture shaken gently with rotary motion to avoid emulsification. The aqueous layer was permitted to settle and was drained away. The fat-ether mixture was then washed with 20-ml. portions of distilled water, the ether evaporated on a hot plate and in a 100° C. oven, and the fat titrated for acidity. Results are presented in table 2.

These data reveal that this procedure of removing fatty acid is highly efficient, with three 20-ml. portions of 0.05 N NaOH being sufficient to reduce the fat titer by more than 90 per cent in a 50-gram sample when the acid degree approximates 3 to 5. However, there appears to be a point of irreducible minimum in the fat acidity and additional washings do not appreciably alter this value. This residual acidity amounts to approximately 0.15–0.30 ml. of 0.05 N NaOH per 10-gm. sample of fat. These values may be due to a slight degree of hydrolysis of the fat or a slight extent of end-point fading occurring during the titration procedure. On the basis of

these results it would appear that three to five washings of fat with small portions of weak alkaline solutions, followed by two rinsings with water, will remove practically all of the free fatty acids, provided that the alkali used is in excess to that required for neutralization by at least 75 per cent. This amount of washing is decidedly less than was used by Hiscox, Harrison, and Wolf (7) for cheese fat.

*Steam distillation of fatty acids.* Steam distillation of fatty acids was conducted using the multiple unit described under "Procedure." In preliminary trials, butyric acid (Eastman) was used for the distillation. Suffi-

TABLE 2  
*Efficiency of removing fatty acids from a fat-ether mixture by washing with a weak NaOH solution*

Trial No.	Size of sample	Amount of ether	Amount of*		Titer of fat†			Volume alkali times volume of fat	Excess of 0.05 N NaOH solution
			Washing with 0.05 N NaOH	Rinsing with water	Original	Final	Reduction		
	gms.	ml.	ml.	ml.			%		ml.
1	50	120	2-20	2-20	7.47	0.83	88.88	0.8	2.65
2	50	120	3-20	2-20	7.46	0.45	93.97	1.2	22.70
3	50	120	4-20	2-20	7.46	0.30	95.98	1.6	42.70
4	50	120	4-20	2-20	6.90	0.23	96.67	1.6	45.50
5	50	120	5-20	2-20	6.90	0.25	96.38	2.0	65.50
6	50	120	5-20	1-20, 2-10	6.90	0.15	97.83	2.0	65.50
7	50	120	6-20	2-20	6.90	0.30	95.65	2.4	85.50
8	50	120	6-20	2-20	6.90	0.20	97.10	2.4	85.50
9	50	120	7-20	2-20	8.60	0.20	97.68	2.8	97.00
10	25	100	8-20	2-20	8.45	0.23	97.28	6.4	138.87
11	25	100	4-20	4-20	8.45	0.20	97.63	3.2	58.87
12	25	120	5-20	4-20	8.45	0.18	97.87	4.0	78.87
13	25	120	4-10	4-10	8.45	0.23	97.28	1.6	18.87
14	25	120	5-10	4-10	8.53	0.25	97.07	2.0	28.67

\* First values indicate number of washings, second values indicate the volume used per washing; thus 2-20 indicates 2 washings of 20 ml. each.

† Titer of fat represents mls. 0.05 N NaOH solution per 10 gms. To change to acid degree divide by 2.

cient butyric acid was used to require 20-25 ml. of 0.05 N NaOH for neutralization. This acid was neutralized with the alkali, then adjusted to pH 2 with  $H_2SO_4$ . The volume was standardized to 200 ml. and steam distillation was conducted. Each 100 ml. of distillate was titrated and corrected for the blank value. Average results secured for seven trials are presented in figure 1, in which a comparison is made with the results of Hiscox and Harrison (7). The two curves exhibit similar trends and indicate that when the volume of distillate is twice the original volume 98 per cent of the acid is recovered. These data also are in agreement with those secured by Dyer (3). Although results are not presented, distillation of n-caprylic acid revealed even more rapid recovery, with approximately 93 per cent being recovered

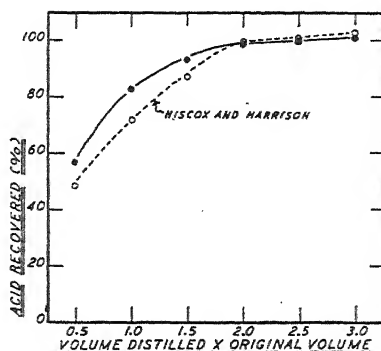


FIG. 1. Rate of steam distillation of n-butyric acid in comparison to rate observed by Hiseox and Harrison.

with distillation of one times the original volume. This compares favorably with the 95 per cent reported by Hiseox and Harrison (7).

Following the standardization of the distillation procedure by use of the pure fatty acids, trials were conducted in which butterfat having high free fatty acid content was steam distilled. In some trials, approximately 150 grams of butteroil were used, in other trials, the size of the sample was approximately 50 grams. The volume of the sample was increased to 200 ml. with acidified water and 200 ml. portions of the distillate were titrated. Results of 7 trials are presented in table 3.

Data in this table reveal that steam distillation of the pure butteroil results in poor recovery of the fatty acids. This may result due to the fact that no appreciable quantity of the lower volatile fatty acids are in the free state, or to the retardation action of the fat. The volatility of 4–6 per cent of the fatty acids is considerably lower than 15 per cent reported by Fouts (4). However, Fouts titrated 200 ml. of distillate from 10 grams of fat, a value representing 20 times the original volume as contrasted to 6 to 10 times the original volume used in the experiments herein reported.

TABLE 3

*Amount of volatile fatty acids recovered by steam distillation of butterfat with high free fatty acid content*

Sample No.	Acid degree of fat	Per cent of total free fatty acid after distilling		
		3 × original vol.	6 × original vol.	10 × original vol.
1	3.62	2.43	5.03	.....
2	4.25	2.10	3.14	4.02
3	4.25	2.28	3.38	4.34
4	4.35	1.84	2.73	3.96
5	4.33	2.35	3.34	4.50
6	6.75	2.35	5.62	.....
7	7.00	2.86	6.12	.....

To study further the volatility of free fatty acids in butterfat, butterfat having previously undergone considerably lipolysis was divided into two lots. One lot was steam-distilled directly. The other lot was dissolved in ether, washed with 0.05 N NaOH solution and then rinsed with distilled water to remove the remainder of the salts. Fifty grams of fat were used, washed with six 20-ml. portions of the alkali, and rinsed with two 20-ml. portions of water. The alkaline washings and the water rinse were combined, adjusted to pH 2 with  $\text{H}_2\text{SO}_4$  and the solution distilled. Two-hundred-ml. distillate portions obtained from the fat and from the alkaline rinsings of the fat were titrated. Acid degrees of the fat used in this study ranged from 3.62 to 7.0. Average results are portrayed in figure 2.

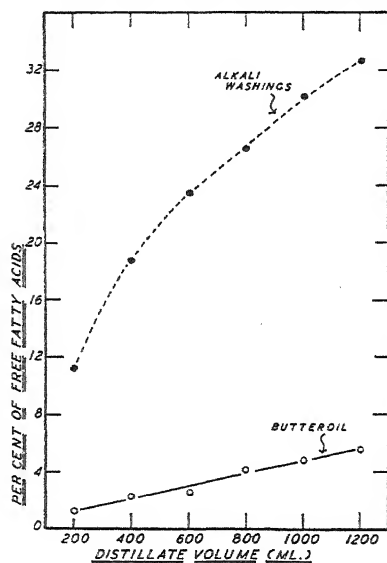


FIG. 2. Recovery of volatile fatty acids from butteroil having high acid degree when steam distillation was conducted on the butteroil and on the alkali washings from the butteroil. (Original volume—200 ml.)

This figure illustrates the remarkable difference in volatility of fatty acid when the acids are removed from the fat and then distilled and when they are distilled directly from the fat. Distillation of six times the original volume resulted in a recovery of only 5.7 per cent of the acids when the distillation involved the fat itself, whereas a recovery of 32.7 per cent resulted when the fatty acids were distilled separately. The rate of distillation of the fatty acids from the acidified-alkaline rinse is also interesting. Approximately 30 per cent of the fatty acids recovered during the total distillation period were recovered by distillation of one times the original volume. However, an appreciable quantity of acids were being distilled at the close of the distillation.

Results secured with the acidified-alkaline rinse indicate that a higher percentage recovery of fatty acids may be obtained from lower acid degree fat. In one trial in which the fat possessed an acid degree of 3.62, 48.8 per cent of the acids were recovered by distillation of seven times the volume, whereas under similar conditions, fat with an acid degree of 7 permitted a recovery of 31.4 per cent. However, additional information is needed in this connection before conclusions are drawn.

#### DISCUSSION

The failure of the washing of the fat with successive portions of warm water to reduce the fatty acid content indicates the possibility that water-soluble fatty acids either are not retained in any appreciable quantity by the fat or are held in such a manner that they are not removed by the water-washing procedure. These findings demonstrate that washing of the butter granules following churning and during the preparation of butteroil for fat titrations will likely have no influence on the resulting titer of the fat. Thus, the preparation of butteroil in lipolytic studies may be greatly expedited by utilizing water to remove the milk-solids-not-fat.

The steam-distillation studies show the undesirability of attempting to measure lipase activity in the milk or cream by steam-distillation of the fat. The proportion of volatile acids obtained by this procedure is so small that differences between samples may not be clearly demonstrated. In contrast, the steam-distillation of acidified-alkaline washings of fat appears to be an excellent method of studying lipolysis. Recovery of the acids was approximately five times as great for the alkaline washings as for the butteroil when the volume of the distillate was six times the original volume.

The high proportion of volatile acids secured when the acids were distilled separately from the fat may serve to indicate that the fatty acids involved are of the water-insoluble fat-soluble type. Hiscox and Harrison (7) found butterfat to distinctly retard the distillation of this type of acid, with the retardation increasing with increasing solubility of the acids in fat. This fat retention effect is especially marked in the results presented herein. In addition, the fact that these acids are not removed by washing fat with water and that the rancid flavor is not retained by the fat also indicates that the free fatty acids present in churned fat are of the fat-soluble type.

#### CONCLUSIONS

The fat acidity of butterfat from homogenized raw milk was not appreciably affected by washing with six successive portions of warm water. However, washing of an ether solution of the fat with a weak alkaline solution is an efficient method of removing the free fatty acids.

Steam distillation of butterfat is highly inefficient as a means of recovering volatile fatty acids, resulting in a recovery of approximately 5 per cent

when distillate volume was six times original volume. In contrast, distillation of acidified-alkaline washings of fat resulted in a recovery of approximately 32 per cent of the free fatty acids.

Free fatty acids in butterfat secured from homogenized raw milk by churning are apparently of the water-insoluble fat-soluble type.

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## THE INFLUENCE OF LOW ENVIRONMENTAL TEMPERATURES ON INTRAMAMMARY TEMPERATURES\*

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Since temperature plays an important part in all physiological processes it is only consistent to expect that variations in the temperature of the udder should affect the production of milk. Although the rectal temperature of the normal cow lies between 100° and 103° F., one would expect the udder, with its comparatively large exposed surface, to be influenced to some extent by the environmental temperature. Many reports have been published dealing with the effect on milk production of low environmental temperatures but no information regarding intramammary temperatures has been found.

Since the teats and lower portion of the udder are more exposed than other portions, one would expect temperature changes in this area to be most marked. Therefore, for the most part, only data on the temperature in the teat and the milk cistern have been used in this study.

Two methods of obtaining the intramammary temperature were used. Inserting a small metal thermometer (Model 226L001, Weston Electric Instrument Company) directly into the teat cistern and making readings as soon as a constant temperature was indicated, was one. Hereafter, this method will be designated as the "direct method." The other method, an "indirect" one, was that of estimating the intramammary temperatures from the temperatures of the first drawn milk.

The direct method of obtaining the intramammary temperature is unquestionably the better method. However, due to the danger of introducing infection into the udder, only cows which were to be discarded for milk purposes could be used in this manner. Of the eight cows on which direct temperature readings were obtained, four were reported by our veterinary department to be mastitic in one or more quarters. However, none of these cows had acute mastitis in the commonly accepted meaning of the term.

Cows with mastitis were kept in a separate barn from the one housing the healthy animals. The removal from the herd at frequent intervals of all cows infected with mastitis as well as the difficulty of carrying out even two trials at the same environmental temperature made it impossible to secure adequate data by the direct method. For these reasons it seemed wise to check the direct method by an indirect method and then use the indirect method to obtain more data from cows free of mastitis.

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During the exposure period, the cows were confined to a small protected yard just south of the barn. Some of the differences noted with the same cow and the same environmental temperature may have been due to differences in activity of the cows from day to day and to wind velocity. Also, when the sun was shining, the environmental temperature tended to fluctuate rapidly. Data showing the effect of repeated sampling, *et cetera*, were obtained in the barn proper after the severe winter weather had passed.

Before securing direct readings each cow was brought into a room whose temperature varied from 65° to 75° F. The udder was washed and disinfected. The thermometer whose stem was seven and three-fourths inches long, was inserted as far as possible into the mammary gland and readings made by means of a mirror held beneath the inverted dial. The dials were graduated in two degree intervals and the thermometers were guaranteed by the makers to be accurate to within one-half of one per cent, which was verified. The order in which readings were taken is indicated in the tables.

In making indirect readings, the udders were quickly washed with chlorine solution and samples collected as soon afterwards as possible. The samples of milk were collected in four test tubes, one for each quarter of the udder. These 60-ml. test tubes were each inserted into pint thermos bottles and securely attached so as to be doubly insulated from the effects of the temperature of the room in which readings were made. Cork stoppers with small thermometers (like those commonly used in the Van Slyke blood gas apparatus) completed the assembly.

Just before milk samples were obtained the thermos bottles were removed from the constant temperature oven and brought to the room where temperature readings were made. Each 60-ml. tube was emptied of water, filled at once with warm milk, preferably from a cow subjected to the same temperature changes as the environmental animals, and the tubes corked. In taking milk samples from experimental animals, the "tempering milk" was discarded and each tube filled at once with milk from a quarter of the udder of an experimental animal. The thermos bottles were held close to the end of the teat and were filled with milk as rapidly as possible. Due to foaming only about 50 ml. of milk were removed at each sampling. The tubes were then corked and the thermos bottles inverted so that milk and not foam was sure to cover the thermometer bulbs. Readings were made as soon as samples from all quarters had been obtained.

## RESULTS

Before attempting to study the effect of low environmental temperatures on the udder of the cow it seemed wise to determine the normal range of udder temperatures. Table 1 not only shows the udder temperatures by the direct and indirect method, but data are also presented on temperatures of each quarter both for healthy cows and those considered to be carriers of

mastitis. These values were obtained after the cows had been in the barn for several hours. Although barn temperatures varied from 58° to 70° F. at different times during the experiment, readings were obtained for all quarters of the udder at any one temperature.

With some cows the temperature of the milk in the quarter sampled last was higher than the milk in the quarter sampled first. This was not consistently true. There was a definite tendency, though, for the milk in the two front quarters to be slightly higher in temperature than the milk in the two rear quarters. There was no regular difference between the intramam-

TABLE 1

*Data showing average intramammary temperature of udder with environmental temperatures of 58° to 70° F.*

Quarters (observations made in order indicated)	No. of observations	Direct method*	No. of observations	Indirect method†
Cows free of mastitis				
		°F.		°F.
1. Right front .....	10	99.1	28	97.9
2. Right rear .....	10	98.7	28	97.6
3. Left rear .....	10	98.7	28	97.4
4. Left front .....	10	99.1	28	97.8
Two front .....	...	99.1	...	97.85
Two rear .....	...	98.7	...	97.5
Cows with mastitis				
1. Right front .....	31	99.0	30	97.6
2. Right rear .....	31	98.7	30	97.9
3. Left rear .....	31	98.5	30	97.2
4. Left front .....	31	99.0	30	97.6
Two front .....	...	99.0	...	97.6
Two rear .....	...	98.6	...	97.5

\* Thermometer inserted into gland cistern.

† Temperature of first drawn milk.

mary temperatures of cows free of mastitis and those known to have been previously infected with mastitis.

The intramammary temperatures secured by the indirect method are about 1.2 degrees lower than those obtained by the direct method. These differences were greater when barn temperatures were low and with cows which milked slowly. The intramammary temperature, as obtained by the direct method, showed no consistent change with the usual fluctuations in barn temperature (58° to 70° F.), so it is apparent that the indirect method becomes less reliable as lower environmental temperatures are studied.

Variations of the intramammary temperature for the same cow varied between trials as much as 1° F. by the direct method and 1.5° F. by the indirect method. Changes in environmental and body temperature caused some of this fluctuation. Washing the udder before collecting the samples

may have produced different responses on different days. Differences in the amount of bedding protecting the udder from the barn floor also varied from day to day and may have caused some minor fluctuations in intramammary temperature.

Although more pendulous udders might be expected to show lower temperatures than the so-called "tight vessel" type, no definite trend was evident at barn temperatures.

The data presented in table 1 have been rearranged in table 2 so as to show whether the breed or the milk volume of the udder influenced the intramammary temperature. No evidence was found to indicate that either factor is important when the environmental temperature is between 58° and 70° F.

TABLE 2

*Effect of milk production and breed on intramammary temperature (indirect method)*

No. and breed of cows in each trial	No. trials	Average daily production			
		> 60 lbs.	25-60 lbs.	< 25 lbs.	All cows
		Temperature*	Temperature*	Temperature*	Temperature*
20 Holstein .....	3	°F. 98.2	°F. 97.9	°F. .....	°F. 98.0
6 Guernsey .....	3	.....	98.0	98.3	98.1

\* Temperature of first drawn milk. Environmental temperature (barn temperature) varied slightly but samples from every cow were taken at each trial.

TABLE 3

*Temperatures of consecutive samples of milk obtained from the same udder\**

Cows used in each trial	Trials	Average temperature of milk from all quarters		
		First sample	Second sample	Third sample
No.	No.	°F.	°F.	°F.
20	3	98.9	98.9	99.1
3	1	98.1	98.7	99.2

\* Data obtained during each trial were taken at the same environmental temperature. The environmental temperature between trials varied from 58-68° F.

In trials, results of which are reported in table 3, a second series of milk samples was taken as soon as the temperatures on the first series of samples had been noted. And then a third series of samples was taken from the same cow, all within about a three-minute interval. During this interval most of the cows had begun to "let down" their milk. Although the last series of samples gave higher values than the first two, the temperature differences were not very important.

Only a very small amount of data bearing on the importance of this "letting down" process were obtained with cows exposed to low environmental temperatures. By the indirect method, readings were obtained on four cows after three hours' exposure at 20° F. (table 4). The udders of

TABLE 4

*Effect of handling the udder on the temperature of the first drawn milk obtained from cows exposed to an environmental temperature of 20° F. for 3 hours*

	Front quarters in °F.	Rear quarters in °F.	All quarters in °F.
Cow No. 1701			
Udder not handled before sample was taken	95.9	95.4	95.6
Cow No. 1044			
Udder washed before sample was taken	97.2	96.8	97.0
Cow No. 1751			
Udder not handled before sample was taken	94.1	92.6	93.7
Cow No. 1756			
Udder washed before sample was taken	97.4	96.7	97.0

two of these cows were washed and disinfected as already noted in the methods of procedure. With the other two cows, milk samples were withdrawn without previous handling of the udder. Although there were always differences in intramammary temperature between cows exposed to the same environmental temperature, the striking differences noted in table 4 would indicate that the handling of the udder is likely to cause the cows to "let down" their milk and that this phenomenon explains why the milk in the udders of these cows tends to "heat up," probably by adding more milk from higher up in the gland to that already present in the milk cistern.

TABLE 5

*Relation between rectal temperature and intramammary temperature when the environmental temperature varied from 58° to 70° F.*

Rectal temperature in °F.†	No. of cows	Total No. of observations	Average intramammary temperature*		
			Fore quarters in °F.	Rear quarters in °F.	All quarters in °F.
100.8	4	24	99.1	98.9	99.0
101.0	3	9	98.7	98.7	98.7
101.1	1	1	98.7	98.8	98.7
101.2	5	25	98.5	98.6	98.6
101.3	3	6	99.2	98.9	99.0
101.4	5	30	99.0	98.8	98.9
101.5	3	12	98.0	97.2	97.6
101.6	4	12	97.8	97.6	97.7
101.7	2	2	97.7	97.3	97.5
101.8	2	4	98.3	98.3	97.3
101.9	3	6	97.7	97.3	97.5
102.0	2	4	98.1	97.9	98.0
102.1	1	1	97.7	97.3	97.5
102.2	1	1	99.4	99.2	99.3
103.1	1	1	99.4	100.0	99.7
103.2	1	1	100.4	100.3	100.4

\* Indirect method.

† Rectal thermometer with extended shank was used so that bulb was inserted two inches deeper than normally.

TABLE 6

*Relation between environmental temperature and intramammary temperature*

Environ- mental temperature in °F.	No. of cows	Total No. of trials	Temperature in °F.			
			Fore quarters	Rear quarters	Average	Rectal
Direct method						
-14	2	1	97.1	96.6	96.9	101.5
-12	1	1	96.2	96.0	96.1	101.4
-10	2	1	95.8	95.3	95.5	101.0
9	2	1	98.2	97.2	97.7	101.3
14	2	2	97.9	96.9	97.3	101.7
16	2	1	98.3	98.0	98.1	101.9
17	2	1	98.3	97.6	97.8	101.6
18	1	2	98.2	97.7	97.9	102.0
19	1	1	98.6	97.7	98.1	101.9
20	2	3	98.0	97.6	97.8	101.7
28	3	2	98.6	97.9	98.2	101.5
29	3	1	98.8	98.8	98.8	101.5
30	2	1	98.7	98.1	98.4	101.6
31	5	1	98.7	97.8	98.2	101.7
32	1	1	98.7	98.3	98.5	101.8
40	1	2	99.0	98.9	98.9	101.7
44	3	1	98.6	97.0	98.1	101.9
48	3	1	98.5	98.3	98.4	101.7
49	2	1	99.2	98.7	98.9	101.3
52	1	2	99.3	99.0	99.1	101.9
54	2	1	99.1	98.7	98.9	101.6
55	2	1	99.0	98.6	98.8	101.4
56	1	1	99.3	99.0	99.1	101.3
58	3	1	99.0	98.6	98.8	101.9
60	2	1	98.8	98.5	98.7	101.6
61	2	1	99.0	98.9	99.0	101.5
65	2	1	99.0	98.5	98.8	101.4
Indirect method						
14	1	1	98.3	97.3	97.8	101.1
16	4	1	96.7	97.6	96.2	101.5
17	3	1	96.4	95.7	96.0	101.6
18	6	1	96.2	95.4	95.8	101.9
19	3	1	96.9	95.8	96.2	101.3
20	4	3	96.7	95.5	96.1	100.9
28	5	2	97.6	97.1	97.3	101.5
29	3	1	97.7	97.5	97.7	101.5
30	4	2	97.7	97.1	96.3	102.1
31	2	1	97.1	96.7	96.9	101.8
32	3	2	97.1	96.3	96.7	101.8
35	5	1	97.5	97.3	97.4	101.4
40	4	2	97.7	97.1	97.4	101.7
48	1	1	97.2	97.0	97.1	101.9
49	1	1	97.9	97.3	97.6	101.6
52	4	2	97.5	97.0	97.2	101.7
56	3	1	97.5	97.2	97.3	101.8
57	6	1	98.8	98.7	98.7	101.3
58	14	1	99.0	98.9	98.9	101.3
68	9	1	99.1	99.3	99.2	101.2
70	21	1	98.6	98.4	98.5	101.4

Since rectal temperatures were always taken at the same time as the temperatures of the udders were being determined, it seemed wise to see if there was any relationship between them. These data, shown in table 5, indicate no relationship between rectal temperature and udder temperature except when the rectal temperature rose above  $103^{\circ}$  F. The samples of milk secured from cows with a high rectal temperature showed a slightly higher temperature than milk from the rest of the herd tested.

The average differences between the rectal and udder temperatures during the winter was  $3.6^{\circ}$  F., by the indirect method, and  $2.7^{\circ}$  F., by the direct

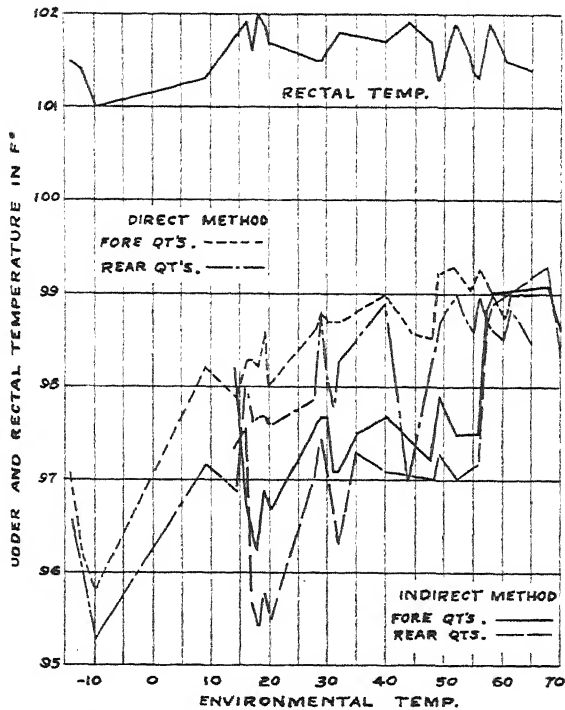


Fig. 1. Relation between environmental, body (rectal) and udder temperatures.

method. The four cows which had a high rectal temperature showed an average difference between rectal and intramammary temperature when measured by the indirect method of only  $3.1^{\circ}$  F.

Most of the information presented so far was obtained with cows housed in a reasonably warm, well-constructed barn. These data give us some idea of the general range of variability in intramammary temperature which may be expected under a moderate environmental temperature. Data are presented in table 6 which show the effect on the udder of external temperatures ranging from  $70^{\circ}$  to  $-14^{\circ}$  F. A total of thirty cows were used in twenty-six trials to obtain this information. Nineteen of these cows were free from mastitis while eleven of them were, or had been, infected with mastitis. The

time of exposure was never less than one hour when direct temperature readings were taken and never less than two hours when indirect readings of udder temperatures were taken. Most of the cows were left outside longer periods unless the weather was very severe.

From the data presented in table 6 and figure 1 it is evident that the intramammary temperature is influenced by low environmental temperatures. During the coldest weather the intramammary temperature was over 5° F. below the rectal temperature when recorded by the direct method. This should be compared with a difference of 2° to 3° F. when the environmental temperature varied between 58° and 70° F. If the cows had been kept quiet and exposed to sub-zero weather, it is quite probable that the difference between the temperature in the teat cistern and in the lower part of the gland cistern, and the rectal temperature, may actually have been as great as 10° F. This is especially true if the animal had lain on the cold ground or snow. The difference in temperature between the fore and rear quarters also became greater as the temperature dropped, probably because of the greater surface exposure of the rear quarters. Differences in rectal temperature as a result of exposure were negligible.

Only one observation was made with a dry cow. This animal was left out of doors for an hour when the temperature was 10° below zero. By the direct method, temperatures of the different quarters were as follows: right front, 96° F., right rear, 93° F., left front, 94° F., and the left rear, 91.9° F. The unusually low readings for the rear quarters may have been caused by obstructions which would not allow the thermometer to be inserted farther than the base of each teat. This cow's udder was very pendulous and the teats large. In spite of this condition she never appeared to suffer from exposure while cows which were milking heavily occasionally had the ends of their teats frosted. In fact, the danger from low temperatures appeared greatest with cows whose udders were full of milk.

#### CONCLUSIONS

When cows were kept in a barn where the temperature varied between 58° and 70° F., the temperatures of their udders (teats and milk cisterns) averaged 2.5° to 3.0° F. lower than their rectal temperature. When cows were exposed to sub-zero weather the observed differences increased to around 5° F. The movement of the cows into the barn and the washing of the udder before inserting the thermometer into the gland cistern decreased the actual difference between rectal and udder temperatures. Otherwise, greater differences would have been observed.

#### ACKNOWLEDGMENT

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# THE COMPARATIVE EFFECTS OF CONTINUAL AND ROTATIONAL SYSTEMS OF GRAZING ON THE CAROTENE CONTENT OF PERMANENT PASTURE HERBAGE AND OF THE MILK PRODUCED THEREFROM\*

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The favorable returns from intensive systems of pasture management in Europe have attracted widespread attention in this country. One of the foremost advantages affirmed for this system is the improvement of the quality of the herbage. Since improved pastures are generally accepted as a practical solution to many difficult nutritional problems in the livestock industry today, any practical measure that reputedly enhances the nutritive value of the pasture plants merits consideration.

Rotational grazing, an essential phase of intensive pasture management, has been reported to increase the carrying capacity of pastures in several cases (9, 10, 19). Conceivably this increased yield could be related to qualitative changes, particularly in carotene values, of the herbage. Since there is a paucity of information on this subject, the need was deemed adequate to warrant investigation.

Thus a determination of the comparative effects of continual and rotational systems of grazing on the carotene of the herbage, primarily, and of the milk, secondarily, constituted a supplementary phase to a three-year study designed to ascertain the applicability of rotational grazing to improved Bermuda grass pastures.

## EXPERIMENTAL

*Pastures.* The grazing areas were improved pastures, previously described (5). The total area of six acres was divided by permanent fences into three two-acre paddocks. Each year one of these was continually grazed while the remaining two, subdivided by temporary electric fences to form a four-unit system, were rotationally grazed. In the course of the three-year investigation each paddock was grazed continually one season and rotationally two seasons.

The flora consisted primarily of hop clover (*Trifolium procumbens* L.) in early spring and Bermuda grass (*Cynodon dactylon* (L.) Persoon) during the remainder of the grazing season. However, small amounts of other

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plants including white Dutch clover (*Trifolium repens* L.), bur clover (*Medicago arasica*) and common vetch (*Vicia sativa*) also contributed to the early grazing. In late spring during the brief interim between the maturation of the legumes and the development of Bermuda grass to the grazing stage and in the latter part of summer when droughts occurred, available herbage was sometimes sparse.

Annual applications of a 6-12-6 fertilizer were made at the rate of 400 pounds per acre during either late winter or early spring.

At various times throughout the grazing season, the paddocks were mowed to remove ungrazed clumps of grass and to check the growth of scattered areas of objectionable weeds.

*Grazing animals.* Lactating cows of the Holstein, the Guernsey and the Jersey breeds were used in comparing continual and rotational grazing. Recommended procedures (1) were followed in the selection, allotment and management of the cows.

The pasture grass was supplemented with a simple low-carotene concentrate mixture, which was fed according to the quantity of herbage available and the amount of milk produced by the individual cows. During periods of scanty grazing, beet pulp also was included in barn feeding.

The frequency of transfer from one unit to another in the rotational system varied with the stage of maturity and the rate of growth of the pasture herbage. Hence, the rotational rate was more rapid in spring than in the latter part of the pasture season. During the annual grazing period, an average of six rotation cycles of 29.5 days each were completed. In a representative cycle each pasture unit was grazed 7.5 days, leaving an interim of 22 days for recovery.

*Herbage samples.* The samples of pasture herbage were composites representing clippings collected at random over the respective pasture areas. The plants were cut at a height approximating the level to which it was grazed by the cows. In the rotational system a sample was taken from each section immediately before the cattle were released onto the area. In the continual system the first sample was gathered shortly preceding the initial grazing, and subsequent samples were collected at the same time as each alternate sample from the rotational series was clipped. This plan resulted in the collection of approximately two samples from the continually grazed area and four from the rotational sections each month; thus the number of samples from the two areas was in the same ratio as the acreage involved.

Each gross sample of collected grass was chopped sufficiently fine to permit accurate sub-sampling for carotene analysis.

The analytical procedure for the determination of carotene was based on the principles set forth by the Association of Official Agricultural Chemists (2) for the preliminary extraction and the modification described by Hegsted *et al.* (7) for the final extraction. The amount of extracted caro-

tene was calculated from colorimetric measurements, using a standardized solution of potassium dichromate as the reference reagent.

*Milk samples.* Individual milk samples for carotene analysis were collected from a representative of each of the three aforementioned breeds in the respective grazing groups. These samples were collected bi-weekly during three consecutive periods, pre-grazing, grazing and post-grazing. The minimum length of the first and last periods was one month, during which time the cattle were barn-fed, receiving corn silage as the only roughage. The duration of the grazing period varied from year to year, depending primarily on climatic conditions.

Each milk sample was a composite of aliquot portions taken from each of the three normal milkings during a day. In the period between milkings the collected milk was stored at a temperature of 35° to 40° F. Within three hours after the final collection, the sample was composited and subjected to the initial analytical procedures.

The fat of the milk was determined by the standard Babcock method and the carotene was measured by a procedure developed by one of the authors (Mitchell) in 1936. The available methods at that time required the use of milk fat as the starting material. These procedures necessitated not only the use of large volumes of milk but also the expenditure of much time and labor to prepare the fat. In searching for a suitable procedure the Roes-Gottlieb (2) method of extracting fat from whole milk was adopted as the base from which the following procedure evolved:

A 50-ml. portion of milk is placed into a 250-ml. separatory funnel. Seven ml. of ammonium hydroxide and 50 ml. of ethyl alcohol (aldehyde-free) are added successively. The contents of the funnel are shaken after each addition. Fifty ml. of ethyl ether and 50 ml. of petroleum ether (b.p. 35–60° C.) are then added in the order listed and mixed gently after each addition. Vigorous agitation may result in emulsification and poor separation of the ether layer. The contents of the funnel are allowed to stand 30 minutes for complete separation after which the lower layer is drawn off and the remaining material reextracted with a mixture of 25 ml. of ethyl ether and 25 ml. of Skelly solve B (petroleum ether, b.p. 60–70° C.). All material from the two extractions is placed into a distillation flask and distilled under reduced pressure at a temperature of 70–80° C. to remove the more volatile solvents. Usually 10 to 15 ml. of a solution, mainly Skelly solve, is left in the flask.

This residual material is transferred to an Erlenmeyer flask where it is mixed with 25 ml. of an alcoholic potassium hydroxide solution (60 gm. potassium hydroxide made up to one l. with ethyl alcohol). The fat present is saponified by refluxing 30 minutes, after which the contents of the flask are cooled and transferred to a separatory funnel by rinsing with 20 ml. of water. The material is extracted twice with 20-ml. portions of Skelly

solve B. The extracts are combined and washed three times with small quantities of distilled water. This washed ether extract is concentrated under partial vacuum to less than 25 ml., after which it is dried over anhydrous sodium sulphate. The extract is then transferred to a glass-stoppered graduated cylinder. Hexane is added to make the volume up to approximately 25 ml., the dilution depending on the intensity of the color needed for comparison.

The carotene content of this solution is determined by means of a spectrophotometer.

Since this procedure was developed, other investigators (13, 17) have employed the Roesse-Gottlieb principle of extraction in determining the carotene of milk and milk products.

*Calculations.* The quantity of carotene in the pasture herbage was calculated as micrograms per gram of dry matter and that for milk, standardized at four-per-cent fat, as micrograms per liter. Since this four-per-cent standard is frequently used in dairy calculations, it was adopted as the common base for expressing the carotene content of the milk produced by the three different breeds.

For each grazing system, the carotene data for the milk from the three breeds and for the pasture herbage were grouped chronologically by semi-monthly periods for each grazing season. Subsequently these data for the three seasons were averaged by the corresponding calendar periods. This organization of the data was designed to show trends ascribable to seasonal changes as well as to indicate variations resulting from the different systems of grazing.

#### RESULTS

The results as shown in the graphical summary of the data, figure 1, are essentially negative; that is, the system of grazing effected no marked and consistent differences in the carotene of either the pasture plants or the milk.

In the case of the carotene concentration of the herbage, the only notable difference that could be related to the system of grazing occurred in June when there was a transition from leguminous plants to Bermuda grass. Since the carotene of plants generally bears a direct relationship to their rate of growth, the observed difference at this period might be ascribed to the fact that the continual grazing retarded the rate of recovery of the pasture herbage to a greater degree than did the rotational grazing.

Irrespective of the system of grazing, the trends and fluctuations in the carotene of the herbage reflected the influences of several interrelated modifying factors, most pronounced of which were stage of maturity and rainfall. When the clovers and vetches were flourishing in the spring and Bermuda grass was in its initial stages of growth in the early summer, the carotene concentrations were higher than at any other time. The effect of maturation, accompanied by droughts in late spring and early fall, was indi-

cated by the interrupted decreases in the Bermuda grass, first, following the initial flush in June and, second, after the marked growth retardation in early fall.

A comparison of the carotene values of the milk produced from the respective systems of grazing disclosed no pronounced differences that might be ascribed to the systems of grazing. This observation is in accord with expectations since the yield (18) and the carotene values of the herbage from the comparative systems were similar.

The trends of the carotene of the milk, for the most part, were typical for normal pasture grazing. The precipitous rise during the first two weeks following the change of the feeding regime from winter roughage to pasture herbage is in agreement with long-established precedent (14), but the subse-

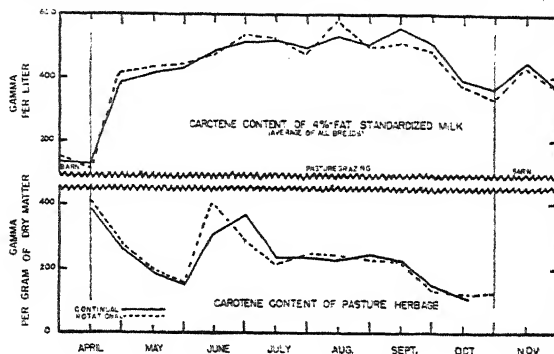


FIG. 1. Summary of a three-year pasture investigation showing the comparative effects of continual and of rotational grazing on the carotene concentration of the pasture herbage and of the milk produced therefrom.

quent retarded rate of increase to the maximum plateau, apparently the "ceiling" value (16), is somewhat unusual (11). Evidently a decrease in the carotene intake resulting from the maturation of the spring legumes was the primary factor contributing to this retardation. A probable secondary operative element was the increased output in the milk; even though the amount of carotene eliminated through this channel constitutes only a small fraction of the total amount ingested (3, 4, 6, 8, 12, 15). The changes in the milk yield during May and June were incommensurate with the reduction in the availability of fresh green herbage. Therefore, the additive results of these relative changes in intake and output would account, in a great measure, for the prolongation of the time necessary to reach equilibrium.

The temporary interruption of the decline of carotene concentration of the milk coinciding with the change from grass to silage might be attributed primarily to an increased intake of carotene. The fresh silage was more palatable and probably more potent in carotene than the Bermuda grass in an advanced stage of maturity.

## DISCUSSION

The emphasis placed on the rôle of carotene as the primary source of vitamin A active substances in dairy cattle nutrition has led to the exploration of a multitude of factors affecting the concentration of this compound in the plants commonly used for dairy feeds. Grazing *per se* probably affects the carotene of permanent pasture herbage to the extent that it modifies the direct influence of other factors. Grazing practices that either impair plant development or permit the plants to reach an advanced stage of maturity obviously adversely affect carotene values.

In the foregoing comparison of continual and rotational systems of management the intensity of grazing was regulated so that optimum returns were obtained from lactating cows throughout the grazing season. Since the carrying capacity of the pastures under the two systems of grazing was approximately the same, the similarity in carotene values was in accord with expectations when these observations are considered in the light of the vital rôle that the quality of forage plays in milk production.

It is reasonable to assume that any pasture providing the quality and the quantity of herbage necessary to maintain optimum milk yields over a prolonged period also would supply carotene in sufficient amounts to raise its concentration in the milk to the "ceiling" value. Irrespective of system of grazing the uniform level of carotene in the milk (figure 1) during the summer months and the lag in its reduction in relation to the initial decrease of carotene in the herbage, during the latter part of summer, tend to support the foregoing assumption. However, a possibility that cannot be disregarded entirely is that the intake-output relationship of the carotene during the summer months might have been so constant that a uniform level in the carotene of the milk was maintained at a "sub-ceiling" level.

During the latter part of the grazing season, the decrease in the carotene of the milk following that of the herbage indicates that either the cows did not have a carotene reserve or that the reserve was not being utilized in sufficient amounts to maintain the prevailing high level in the milk. This last suggestion appears to be the more rational explanation in view of the present knowledge of depletion of body stores.

Even though the observations of this investigation disclosed no significant differences that might be ascribed to the system of grazing, the results do not preclude the possibility that the two systems may reveal a different relationship, particularly in the carotene of the herbage, with other species of plants and/or different soils and fertilizers.

## SUMMARY

A study was made of the relation of continual and rotational systems of grazing to the carotene content of the herbage of improved Bermuda grass

pastures and of the milk produced by respective groups of cows grazing thereon.

The system of grazing effected no marked and consistent difference in either the concentrations or the variations of the carotene in the herbage.

The carotene concentrations and fluctuations of the milk revealed no differences that could be ascribed to the system of grazing.

The quantitative changes in the carotene ingested were reflected in the carotene values of the milk during the spring and the fall when the concentrations in the milk were at a "sub-ceiling" level, but this did not hold true during the summer, since the values of the milk apparently were at "ceiling" level.

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# OXIDIZED FLAVOR IN MILK. XIII. STUDIES OF CUPRIC COMPLEXES OF ASCORBIC ACID AND CERTAIN AMINO ACIDS AND THEIR POSSIBLE RELATIONSHIP TO OXIDIZED FLAVOR DEVELOPMENT IN MILK\*

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In studies on the mechanism for the production of oxidized flavor in milk Olson and Brown (4) postulated that the ascorbic acid in milk was oxidized by copper ions giving hydrogen peroxide as a product; the hydrogen peroxide was then thought to oxidize the phospholipides of milk, producing the oxidized flavor. It was found, however, that excess ascorbic acid prevented the formation of oxidized flavor. This would seem to be an anomaly. However, this might be explained if ascorbic acid formed a complex ion with copper.

Wark (7), in studying the complex ions of hydroxy acids with copper, stated: "The capability of any hydroxy-compound to form complexes with metallic oxides must depend on the acidity of the hydroxyl group. The more strongly this group functions as an acid, the more stable will the complexes be." From this it would seem that the highly acidic hydroxy group of ascorbic acid could form a complex.

Morton (3) reported that in neutralized solutions copper complexes were formed with tartaric, citric, malic, and salicylic acids. In alkaline solution these complexes were destroyed with the formation of highly basic hydrosols. In contradiction to Wark (7) Morton further stated that no stable copper complexes were formed with glyceric, lactic, glycollic, or mandelic acids, the apparent stability of the cupric salts of these hydroxy acids in the presence of caustic alkalies being due to the peptizing properties of the hydroxy-acid anion.

Any compound that forms a copper complex would help prevent the oxidation of the normal ascorbic acid in milk and thus inhibit the formation of oxidized flavor. Sherwood and Hammer (5) have reported concentrations of citric acid salts ranging from 0.07 per cent to 0.33 per cent in milk. In addition, the proteins of milk should form copper complexes although there is some dispute as to the type of complex formed.

Proteins may form complexes with copper through the free amino groups. Thus they would act similarly to amino acids. Borsook and Thimann (1)

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studied the equilibrium relations existing in solution between copper ions and glycine and alanine. They were studied by measurement of absorption spectrum and by copper electrode potentials through a range of hydrogen ion concentrations from pH 0 to 13. They found four types of complexes of both glycine and alanine, depending on the pH of solution.

Smythe and Schmidt (6) studied the formation of complexes of sixty-one compounds with iron. This would be similar to the complexes of copper. By calculating the residual negative charge of the nitrogen atom in amino acids, they reasoned that monoamino monocarboxylic acids would not form a complex. Their experimental work confirmed this. However, they found that dicarboxylic acids and hydroxy acids did form complexes. In addition they claimed the formation of a complex with phosphoric acid and iron due to the residual negative charges on the hydroxy groups of phosphoric acid. The formation of an iron complex with casein was claimed to be due to the phosphoric acid in casein. These studies were done at pH 2.5.

The purpose of the present study was to determine whether or not copper complexes were formed with ascorbic acid and amino acids. Citric acid was also used for comparative purposes.  $\beta$ -alanine was used to compare the action of  $\alpha$ -amino and  $\beta$ -amino acids.

#### EXPERIMENTAL

Most determinations of copper complexes are done with relatively high concentrations of copper. In this work it was desired to work in the range of copper concentration that might be found in milk, that is, about one part per million. For this purpose a method for determining very small amounts of ionized copper was sought. A modified Biazzo (2) method was found applicable. By using a 10-centimeter tube in a Keuffel and Esser color analyzer at a wave length of 450 m $\mu$ ., a range of copper concentration from 0.01 mg. to 0.1 mg. was accurately determined. The copper content of the water and reagents was estimated by using a water blank with the color reagents against the different copper concentrations. By plotting  $-\log T$  against concentration of copper a straight line parallel to the line obtained by running the various copper concentrations against a chloroform blank was obtained. From the distance between the lines the copper content of the reagents and water was found to be from 0.01 mg. to 0.015 mg. for the various batches of redistilled water used.

Although it was desirable to determine the amount of copper ionized at pH 6.5 to 7.0, this method (2) used acetic acid for the development of color and, consequently, the amount of copper ionized was actually determined at a pH of about 3.5. According to Morton (3) the complex of copper should be determined at neutrality. Ascorbic acid and citric acid with copper showed a diminution of copper ions, but these values would have to be considered minimum values. The amino acids showed very little diminution

of copper ions at the pH of reaction. Since Borsook and Thimann (1) have shown that a different complex of amino acids is formed at pH 3.5 than at pH 6.5, this method was not used for the determination of the amino acid complexes.

The amount of ionizable copper with citric acid was determined by adding concentrations of 0.1 per cent to 1.0 per cent of citric acid neutralized to pH 6.5, to 0.1 mg. of copper and adding the reagents to develop color to a total volume of 100 ml. The color was extracted from the water solution with 25 ml. of chloroform, the chloroform was centrifuged for a few min-

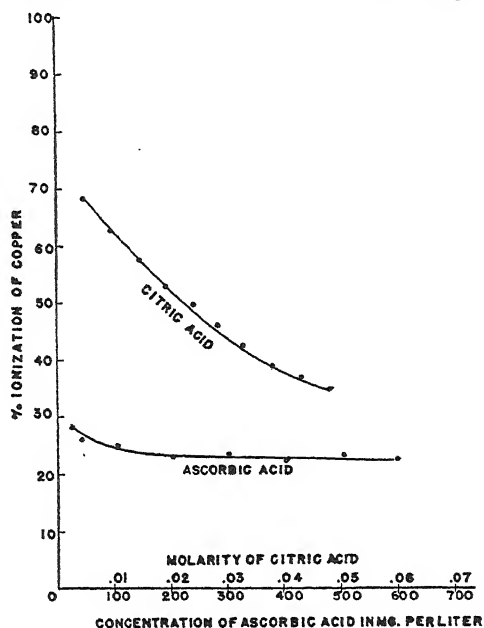


Fig. 1. Ionization of copper with citric and ascorbic acids.

utes to separate water droplets, and the color was immediately read in the color analyzer.

In a similar way the ionization of copper was determined with ascorbic acid, using concentrations of 20 mg. to 600 mg. per liter.

The results of both the citric acid and the ascorbic acid are shown in figure 1. They are calculated as the percentage of the total copper ionized. The curve for citric acid shows results typical for a complex, that is, the higher the concentration of citric acid the less copper was ionized. The ascorbic acid shows an entirely different type of curve. The amount of ionizable copper remains practically the same for all the concentrations of ascorbic acid. Instead of a typical complex like that of citric acid, the ascorbic acid seems to form a definite compound of copper ascorbate. The ascorbic acid ties up all but about 25 per cent of the copper very definitely.

It was desired to see what effect the copper complex of citric acid had on the protection of ascorbic acid. In a preliminary trial it was found that copper in a concentration of one part per million in 13 minutes completely destroyed, 40 mg. per liter of ascorbic acid in a 0.05 M. phosphate buffer of pH 6.8. This 13-minute period was therefore used as a comparative time interval. A series of 100-ml. volumetric flasks containing 0.1 mg. copper, the phosphate buffer at pH 6.8 and neutralized citric acid in concentrations of 0.1 per cent to 1.0 per cent was set up. Ascorbic acid to make a concentration of 40 mg. per liter was added, the flask filled to volume quickly,

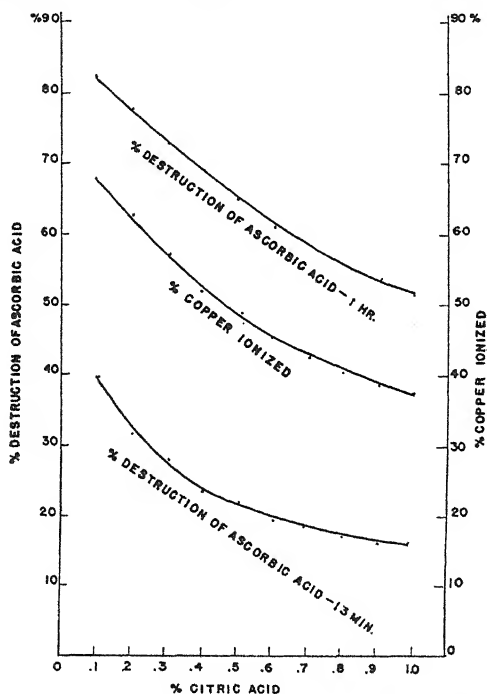


Fig. 2. Ionization of copper and destruction of ascorbic acid (40 mg./l.) with citric acid.

mixed, and placed in the dark. After exactly 13 minutes an aliquot was removed and titrated with a solution of sodium 2,6-dichlorobenzenoneindophenol to determine the amount of ascorbic acid remaining. A similar aliquot was titrated after one hour.

The results are shown in figure 2. For comparison the curve for ionizable copper with citric acid is also shown. It is readily seen that less ascorbic acid is destroyed with increasing concentration of citric acid. The citric acid protects the ascorbic acid from destruction. In addition the two curves for the destruction of ascorbic acid are similar in shape to the curve for ionizable copper, particularly the curve for one hour. These curves

show definitely that the inhibition of ascorbic acid destruction is due to the removal of copper ions by the citric acid complex.

The same method was used to determine whether or not amino acids prevent the destruction of ascorbic acid by copper. The experiments were conducted exactly the same as for citric acid, using glycine, glutamic acid, alanine, and  $\beta$ -alanine in the same molar concentrations. Thus results were obtained for two monoamino monocarboxylic amino acids, one monoamino dicarboxylic acid, and one  $\beta$ -amino acid. Citric acid was also run for comparative purposes. The results are the average of two or three determinations.

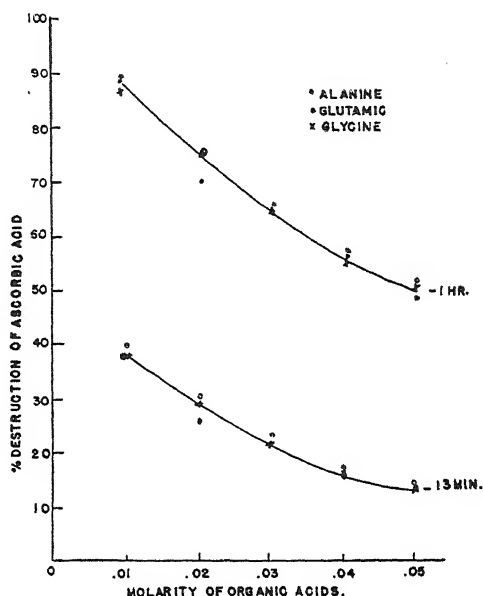


FIG. 3. Protection of ascorbic acid by complex ions of  $\alpha$ -amino acids.

The results of the amino acids as inhibitors of the destruction of ascorbic acid are shown in figures 3 and 4. It will be noted in figure 3 that glycine, glutamic acid, and alanine all fall along the same curves. The variation from the curves is no more than the experimental error in the individual determinations. It may be noted in figure 4 that citric acid forms a curve of different shape from the amino acids (glycine, glutamic, and alanine) but of close to the same protective value. Glutamic acid gives the same curve as glycine and alanine, contrary to Smythe and Schmidt's (6) hypothesis that the two carboxyl groups form the complex and not the amino group. Furthermore it must be remembered that the amino acids are compared to the destruction in phosphate buffer which completely destroys the ascorbic acid in thirteen minutes. Smythe and Schmidt's (6) contention that phosphoric acid forms a copper complex is not supported by these results.

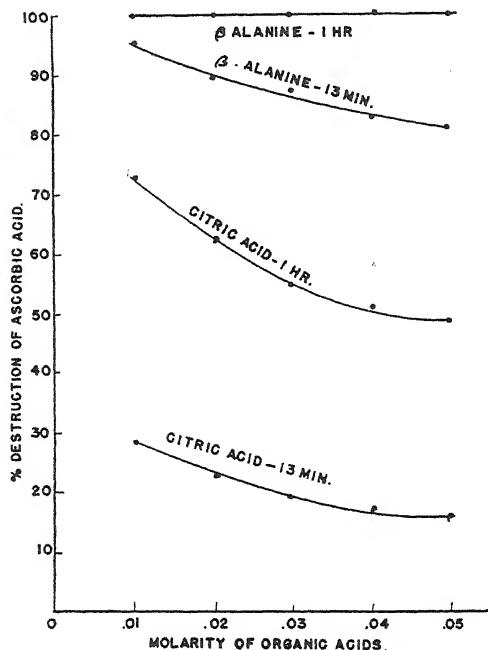


FIG. 4. Protection of ascorbic acid by complex ions of citric acid and  $\beta$ -alanine.

Finally the curves in figure 4 for  $\beta$ -alanine show that the amino group in the  $\beta$  position forms a very weak complex. After one hour the ascorbic acid is totally destroyed regardless of the concentration of  $\beta$ -alanine. These results would definitely indicate that amino acids form copper complexes through the amino group.

TABLE 1

*The effect of varying amounts of citric acid on the development of oxidized flavor in buffered washed cream containing 42 mg. per liter of ascorbic acid and 1.0 ppm. copper*

	Date					
	8/19/42	8/20/42	8/21/42	1/9/43	1/12/43	1/15/43
Control .....	ox	ox	ox	ox	ox	ox
Washed cream						
plus citric acid						
0.05% .....	.....	.....	ox	.....	.....	.....
0.1% .....	ox	ox	ox	.....	.....	.....
0.2% .....	?	sl. ox	sl. ox	ox	ox	sl. ox
0.3% .....	.....	.....	?	.....	.....	.....
0.4% .....	.....	.....	—	.....	.....	.....
0.5% .....	—*	?	—	—*	sl. ox	—
0.6% .....	.....	.....	.....	.....	.....	.....
0.7% .....	—*	—*	.....	.....	.....	.....
0.8% .....	.....	.....	.....	.....	.....	.....
0.9% .....	—*	—*	.....	.....	.....	.....
1.0% .....	—*	—*	.....	—*	—	.....

Meaning of symbols: \* bitter from citrate; ox oxidized flavor; — no oxidized flavor.

TABLE 2

*The effect of citric acid on the development of oxidized flavor in susceptible milk contaminated with 1.0 ppm. copper*

	Date				
	1/8/43	1/14/43	1/18/43	1/19/43	1/20/43
Control .....	ox*	ox	ox	ox	ox
Milk plus citric acid 0.02 M.	—	—	—	—	—

\* For meaning of symbols, see table 1, footnote.

Since citric acid has been shown to have a protective action against the oxidation of ascorbic acid by the copper ion it seemed desirable to determine its effect on the development of oxidized flavor in washed cream and milk. Accordingly, samples of washed cream were prepared and tested according to the method of Olson and Brown (4). The results of these experiments

TABLE 3

*The effect of adding alanine and glycine on the susceptibility of washed cream containing 42 mg. per liter of ascorbic acid and 1.0 ppm. copper to the development of oxidized flavor*

	Date				
	12/31/42	1/9/43	1/12/43	1/15/43	1/19/43
Control .....	ox*	ox	ox	ox	ox
Conc. of alanine					
0.02 M. ....	ox	ox	sl. ox	sl. ox	ox
0.05 M. ....	sl. ox	sl. ox	sl. ox	—	sl. ox
0.10 M. ....	.....	?	—	—	v. sl. ox
Conc. of glycine					
0.02 M. ....	ox	ox	ox	sl. ox	sl. ox
0.05 M. ....	sl. ox	sl. ox	sl. ox	sl. ox	?
0.10 M. ....	.....	?	v. sl. ox	—	—

\* For meaning of symbols, see table 1, footnote.

are shown in tables 1 and 2. These results confirm the finding that citric acid has an inhibitory effect on the development of oxidized flavor by ascorbic acid.

Likewise, the effect of alanine and glycine was studied on both washed cream and milk. These results are shown in tables 3 and 4.

TABLE 4

*The effect of adding alanine and glycine on the susceptibility of milk containing 1.0 ppm. of added copper to the development of oxidized flavor*

	Date				
	1/8/43	1/14/43	1/18/43	1/19/43	1/20/43
Control .....	ox*	ox	ox	ox	ox
Conc. of alanine					
0.02 M. ....	—	.....	.....	.....	.....
0.05 M. ....	—	.....	.....	.....	.....
Conc. of glycine					
0.02 M. ....	.....	—	—	—	—
0.05 M. ....	.....	—	—	—	—

\* For meaning of symbols, see table 1, footnote.

Here, likewise, both alanine and glycine are found to have an inhibitory action on the development of oxidized flavor. This inhibitory action was not as strong as in the case of the citric acid.

#### DISCUSSION OF RESULTS

These results indicate that the development of oxidized flavor in milk is closely associated with the ionization of copper and its destruction of ascorbic acid. Apparently anything which decreases the ionization of copper will in turn retard the destruction of ascorbic acid and in this manner tend to retard oxidized flavor development. As a result of these findings, it seems that many individual factors may have a bearing on the susceptibility or nonsusceptibility of milk to the development of oxidized flavor.

#### CONCLUSIONS

1. Ascorbic acid forms either a complex or a direct compound with copper ions.

2. The protective action of citric acid on the oxidizing action of copper ions with ascorbic acid is due to the formation of a copper complex with citric acid thus removing copper ions from the reaction.

3. Through the indirect evidence of inhibition of ascorbic acid destruction with copper ions, it is shown that the amino acids—glycine, glutamic acid, and alanine—are equally effective in forming complex ions. With the evidence that  $\beta$ -alanine forms a very weak complex, this shows that the amino acids form copper complexes through the free amino group.

#### ACKNOWLEDGMENT

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## OXIDIZED FLAVOR IN MILK. XIV. A POSSIBLE MODE OF ACTION OF INHIBITORS IN PREVENTING THE DEVELOPMENT OF OXIDIZED FLAVOR IN MILK\*

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Various substances have been found to inhibit the formation of oxidized flavor in milk. Dahle and Palmer (5) found that excess ascorbic acid protects against oxidized flavor. Anderson (1) reported that a pancreatic enzyme added to milk just before pasteurization protects the milk against oxidized flavor. Corbett and Tracy (4) found that both tyrosine and the butyl ester of tyrosine prevented the flavor, and that "Enzylac" and Avenex gave protection. Even ordinary raw sugar gave some protection. Williams and Burgwald (16) found that carotene did not protect, but that mixed tocopherols gave a protection. Garrett (7) found that divalent manganese protected milk against oxidized flavor. Archibald (2) fed an ounce of manganese sulphate daily to cows and analyzed the milk. The manganese content of the milk was doubled, but even this amount was not sufficient manganese to prevent oxidized flavor. Brown and Olson (3) found that potassium iodide added directly to the milk protected against oxidized flavor.

During the summer of 1942 the authors attempted to isolate the active inhibitor in summer milk. The casein was precipitated at the isoelectric point. This was made into calcium caseinate by the method of Palmer (13). The calcium caseinate, whey, and sterilized whey, were then tested on washed cream according to the method of Olson and Brown (12). In most cases the oxidized flavor was prevented. In addition lactose was tried and found to inhibit oxidized flavor. Thus it was found that a number of naturally occurring substances in milk inhibit oxidized flavor.

Brown and Olson (3) proposed a chemical theory for the development of oxidized flavor in milk. This theory postulated that cupric copper oxidizes the ascorbic acid in milk to dehydroascorbic acid with the production of copper in the cuprous state of ionization. The cuprous copper is then oxidized back to the cupric form by dissolved oxygen with the production of hydrogen peroxide. The hydrogen peroxide in turn oxidizes the phospholipides in milk to give the oxidized flavor.

The purpose of this paper was to study the natural inhibitors in milk as well as the inhibitors proposed by other workers in order to test this theory

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of oxidized flavor development. To test this the inhibitors were tried again on milk and wherever possible on washed cream to be sure that a preventive for oxidized flavor was actually present. A chemical explanation was then attempted for the inhibiting action.

Referring again to the theory of the development of oxidized flavor, it is readily seen that it can be prevented (I) by removal of copper ions so that the ascorbic acid is not oxidized. A class of inhibitors in this group are those that form complex ions with copper thus reducing the copper ions in solution. A second class (II) would include substances that are themselves oxidized by hydrogen peroxide before the hydrogen peroxide oxidizes the phospholipides of milk. Because of a lack of knowledge of the chemistry involved, a third class (III) must be added to include substances that inhibit oxidized flavor but for which the mechanism is not understood.

#### EXPERIMENTAL RESULTS

I. The first class of inhibiting substances in which the ionization of copper is repressed is typical of salts of citric acid and amino acids as cited by Olson and Brown (11). They found that hydroxy organic acids form a complex with copper which inhibits the ionization. This in turn inhibits the oxidation of ascorbic acid and consequently the oxidized flavor formation. The effect of amino acids was tried in order to gain an insight into the action of proteins. From the results with amino acids it is seen that the inhibiting effect of proteins would be caused by the free amino and carboxyl groups of the proteins.

*Casein.* Calcium caseinate, made according to the method of Palmer (13), was tried as an inhibitor on washed cream according to the method of Olson and Brown (12). The results are shown in table 1. These results show that calcium caseinate in a three per cent solution protects washed cream against oxidized flavor. In two cases oxidized flavor was inhibited by a percentage lower than the casein content of milk. This would indicate that in the preparation of the calcium caseinate, it was partly hydrolyzed and the liberated free amino groups inhibited the oxidized flavor.

*Proteolytic enzymes.* Since "Enzylac" includes a proteolytic enzyme, the inhibiting effect of this product may be due to a partial hydrolysis of milk proteins which liberate free amino groups to form complex ions with copper. That "Enzylac" does protect milk against oxidized flavor is shown in table 2 where the enzyme was added according to the directions of the manufacturer.

*Ascorbic acid.* Another substance belonging to this class is ascorbic acid, as shown by the results of Olson and Brown (11). The presence of excess ascorbic acid prevents the ionization of copper probably through the formation of a copper compound. In this manner the ascorbic acid differs from the amino acids which form complexes as indicated by decreased ionization of copper with increased concentration of the amino acids.

TABLE 1

*The effect of various concentrations of calcium caseinate on the susceptibility of washed cream containing 42 mg./liter of ascorbic acid to oxidized flavor*

Date	Control Washed cream ppm. Cu				3% calcium caseinate plus ppm. Cu				2% calcium caseinate plus 1.0 ppm. Cu	1% calcium caseinate plus 1.0 ppm. Cu
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5		
7/30/42	ox*	ox	ox	ox	—	—	—	—	.....	.....
8/12/42	sl. ox	ox	ox	ox	—	—	sl. ox	ox	.....	.....
8/14/42	ox	ox	ox	ox	—	—	—	—	.....	.....
12/30/42	ox	ox	ox	ox	.....	.....	sl. ox	.....	sl. ox	sl. ox
1/15/43	ox	ox	ox	ox	.....	.....	—	.....	—	—
1/19/43	ox	ox	ox	ox	.....	.....	—	.....	—	—

\* Meaning of symbols: ox, oxidized flavor; —, no oxidized flavor.

II. The second class of substances includes those which inhibit oxidized flavor by the substances themselves being oxidized by hydrogen peroxide such that the phospholipides of milk are not oxidized. Lactose, avenex, potassium iodide, and manganese showed very little or no protection of ascorbic acid against oxidation when tried according to the method used by Olson and Brown (11) and therefore do not form complexes with copper. Tyrosine showed a very slight effect in 0.5 per cent suspension but no more than could be attributed to the small amount of amino acid present in solution.

*Tyrosine.* The effect of tyrosine as an inhibitor of oxidized flavor both with washed cream and milk is shown in table 3. The results show that 0.1 gram of tyrosine per half pint inhibits oxidized flavor in both milk and washed cream although it may not entirely eliminate the flavor. Since tyrosine is quite insoluble, no higher concentration could be tried. The action of tyrosine as an inhibitor is explained by Mystkowski (10). He has shown that tyrosine added to a solution of ascorbic acid and copper inhibits oxygen evolution and the tyrosine changes to a reddish brown color corresponding to quinone products of tyrosine oxidation. Thus it is seen that tyrosine inhibits oxidized flavor in milk by removing the hydrogen peroxide

TABLE 2

*The effect of "Enzylac" as an inhibitor of oxidized flavor on susceptible milk*

Date	Control milk				Milk + "Enzylac"			
	ppm. Cu				ppm. Cu			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
1/11/43	—	ox	ox	ox	—	—	—	—
1/14/43	—	ox	ox	ox	—	—	—	—
1/19/43	—	ox	ox	ox	—	—	—	—
1/20/43	—	—	ox	ox	—	—	—	—

For meaning of symbols, see table 1, footnote.

formed in the ascorbic acid oxidation. Avenex, being a natural product mixture, might contain some quinone type compound which could prevent oxidized flavor similar to tyrosine.

*Manganese.* It would seem to be very strange that divalent manganese would be an inhibitor of oxidized flavor. However, the results in table 4 show that manganese in sufficient quantities definitely prevents oxidized flavor in milk. The following explanation seems possible. When sodium hydroxide is added to a divalent manganese salt a light tan precipitate immediately forms. On exposure to air for a few minutes the top of the precipitate turns black with the formation of  $\text{MnO}(\text{OH})_2$  due to oxidation by atmospheric oxygen. Similarly if a manganese salt is added to a citrate buffer at a pH of 6.5–7.0, a very light-colored solution results. On standing

TABLE 3

*The effect of the addition of 0.1 gram of tyrosine per half-pint on the development of oxidized flavor in milk and washed cream*

Date	Milk								Washed cream (buffered)		Tyrosine
	Control				Milk + 0.1 gr. tyrosine				Control	Washed cream + tyrosine	
	ppm. Cu				ppm. Cu				1.0 ppm. Cu	Plus 42 mg./l. ascorbic acid 1.0 ppm. Cu	
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5			
12/31/42	—*	—	—	—	.....	.....	.....	.....	ox	ox	0.01 M
1/ 8/43	—	ox	ox	ox	—	—	—	—	.....	.....	.....
1/12/43	.....	.....	.....	.....	.....	.....	.....	.....	ox	ox	0.1 gr.
1/14/43	—	ox	ox	ox	—	—	—	sl. ox	.....	.....	.....
1/15/43	.....	.....	.....	.....	.....	.....	.....	.....	ox	—	0.1 gr.
1/18/43	—	—	ox	ox	—	—	—	—	.....	.....	.....
1/19/43	—	—	ox	ox	—	—	—	—	.....	.....	.....
1/20/43	—	—	ox	ox	—	—	—	—	.....	.....	.....

\* For meaning of symbols, see table 1, footnote.

in the air this gradually becomes a dark brown, indicating oxidation by atmospheric oxygen. Thus manganese either protects milk from oxidized flavor by absorbing the hydrogen peroxide in the ascorbic acid oxidation process, or it may even absorb the dissolved oxygen in the milk thus preventing the oxidation of the copper to form hydrogen peroxide.

*Sugars.* Lactose shows some inhibition of oxidized flavor as is seen in table 5. Since lactose does not form a complex ion with copper, the only possible explanation for this effect is that the potentially free aldehyde group in lactose is oxidized by hydrogen peroxide. To test this further, sucrose was tried on washed cream. Sucrose does not have a potential aldehyde group and it did not inhibit oxidized flavor. Glucose on the other hand is a simple sugar with a potential aldehyde group. As shown in table 6, glucose did tend to prevent oxidized flavor. That glucose is capable of being oxidized by hydrogen peroxide was shown by Witzemann (17) who found that glucose could be completely oxidized to carbon dioxide and water by

TABLE 4

*The effect of various concentrations of manganese on the susceptibility to oxidized flavor of milk and washed cream contaminated with copper*

Date	Milk									
	Control + Cu				Control + 1.5 ppm. Cu + Mn					
	ppm. Cu				ppm. Mn					
	0.0	0.5	1.0	1.5	0	1	2	3	4	5
1/ 8/43	—*	ox	ox	ox	ox	—	—	—	.....	.....
1/14/43	—	ox	ox	ox	ox	ox	—	—	.....	.....
1/18/43	—	sl. ox	sl. ox	ox	ox	—	—	—	.....	.....
1/19/43	—	—	ox	ox	ox	ox	—	—	.....	.....
1/20/43	—	—	ox	ox	ox	—	—	—	.....	.....
Washed cream (buffered) containing added ascorbic acid, 42 mg./l.										
					Control + 1.0 ppm. Cu + Mn					
					ppm. Mn					
					0	1	2	3	4	5
	.....	.....	.....	.....	ox	ox	ox	sl. ox	.....	.....
1/ 8/43	.....	.....	.....	.....	ox	ox	ox	sl. ox	v. sl. ox	.....
1/12/43	.....	.....	.....	.....	ox	ox	sl. ox	—	—	—
1/15/43	.....	.....	.....	.....	ox	ox	sl. ox	—	—	—

\* For meaning of symbols, see table 1, footnote.

hydrogen peroxide in the presence of disodium phosphate in a pH range of 5.9 to 7.35. If this is the case, it would seem to be easy for the small amount

TABLE 5

*The effect of added lactose on the susceptibility of oxidized flavor in milk and washed cream*

Date	Control milk				Milk + 2½% lactose				ppm. Cu			
	ppm. Cu				ppm. Cu				Milk + 5% lactose			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
1/ 8/43	—*	ox	ox	ox	—	—	—	—	.....	.....	.....	.....
1/14/43	—	ox	ox	ox	.....	.....	.....	.....	—	—	—	—
1/18/43	—	—	ox	ox	.....	.....	.....	.....	—	—	—	—
1/19/43	—	—	ox	ox	—	—	sl. ox	sl. ox	.....	.....	.....	.....
1/20/43	—	—	ox	ox	.....	.....	.....	.....	—	—	—	sl. ox
<div> <div>Washed cream + 42 mg./l. ascorbic acid</div> <div>Washed cream + 32 mg./l. ascorbic acid + 2½% lactose</div> <div>Washed cream + 42 mg./l. ascorbic acid + 5% lactose</div> </div>												
8/20/42	ox	ox	ox	ox	—	—	—	—	—	—	—	—
8/21/42	ox	ox	ox	ox	—	—	—	—	—	—	—	—
1/ 9/43	ox	ox	ox	ox	.....	.....	ox	.....	.....	.....	ox	.....
1/12/43	ox	ox	ox	ox	.....	.....	sl. ox	.....	.....	.....	sl. ox	.....
1/15/43	ox	ox	ox	ox	.....	.....	ox	.....	.....	.....	—	.....
1/19/43	ox	ox	ox	ox	.....	.....	sl. ox	.....	.....	.....	v. sl. ox	.....

\* For meaning of symbols, see table 1, footnote.

of hydrogen peroxide formed in the oxidation of ascorbic acid in milk to be used to oxidize glucose or lactose and thus protect from oxidized flavor. That the lactose in milk does not entirely prevent oxidized flavor would be due to both oxidation processes going on at the same time.

The protection from oxidized flavor by tocopherols as found by Williams and Burgwald (16) would be included in this second class of inhibitors also. The tocopherols have a hydroquinone type of structure and are very easily oxidized. The problem as an antioxidant for milk would be to get the tocopherols, which are oil soluble, into a sufficiently fine emulsion to give enough surface for antioxidant properties.

TABLE 6

*The effect of glucose on the susceptibility of milk and washed cream to oxidized flavor*

Date	Control milk				Milk + 0.5% Glucose				Milk + 1.0% Glucose				Milk + 2.0% Glucose			
	ppm. Cu				ppm. Cu				ppm. Cu				ppm. Cu			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
1/18/43	—*	—	ox	ox	—	—	—	—	—	—	—	—	—	—	—	—
1/19/43	—	—	ox	ox	—	—	—	ox	—	—	—	ox	—	—	—	?
1/20/43	—	—	ox	ox	—	—	—	?	—	—	—	—	—	—	—	—
	Washed cream + 42 mg./l. asc. acid No glucose				Washed cream + 42 mg./l. asc. acid 2½% glucose				Washed cream + 42 mg./l. asc. acid 5% glucose							
	ppm. Cu				1.0 ppm. Cu				1.0 ppm. Cu							
	0.0	0.5	1.0	1.5												
1/19/43	ox	ox	ox	ox	sl. ox				sl. ox							

\* For meaning of symbols, see table 1, footnote.

In connection with this second class of inhibitors, there is also a possibility that an inhibitor may directly oxidize ascorbic acid and thus eliminate the oxidation step by copper. In this way hydrogen peroxide would not be formed and no oxidized flavor in milk would develop. Heard and Welch (8) have found such a system in effect in the adrenal glands. They found that the ascorbic acid directly reduced oxidized adrenaline, forming reduced adrenaline and dehydro-ascorbic acid.

III. A miscellaneous class of inhibitors, which includes those that do not fit well into either group one or group two, should also be considered. In this group are the halides. Brown and Olson (3) found that potassium iodide inhibited oxidized flavor to some extent. Mapson (9) found that the halides—iodides, bromides, and chlorides—showed a protective action on ascorbic acid at pH 2.5 but very little at pH 6 to 7. Mystkowski (10) found good inhibition by sodium chloride in concentrations of 4 per cent to 10 per

cent even at pH 7. This may explain why salted cream used for butter does not develop oxidized flavor. The mechanism of this protective action of the halides is not known. Mapson (9) has postulated a complex ion of the halide with copper. However, the authors were unable to show any diminishing of the ionization of copper in a solution of potassium iodide, using the method of Olson and Brown (11).

Substances that prevent oxidized flavor by themselves being oxidized by the hydrogen peroxide are of interest. These substances in order to be effective must have an oxidation-reduction potential such that they will be oxidized before the phospholipides. Tyrosine has such an action, but (tyrosine) is not sufficiently soluble in milk to completely prevent the development of oxidized flavor. Divalent manganese, as was found by Garrett (7), inhibited oxidized flavor. An explanation of this is the oxidation of manganous ions to manganic ions by the hydrogen peroxide. That lactose could inhibit oxidized flavor would seem unlikely but the oxidation of the aldehyde group of the sugar and even further oxidation has been shown by Witzemann (17) with glucose.

Since the proteins, citrates, and lactose in milk all have an inhibiting effect on oxidized flavor, it is natural to expect that an increase in any or all of these substances would show an inhibiting effect on winter milk. The work of Russell and Dahle (15) in which added milk solids in the form of condensed milk or dried milk inhibited oxidized flavor development, may be explained on this basis. However, since the condensed milk and dried skimmilk may have been subjected to a sufficiently high temperature to liberate sulfhydryl groups, these also might explain the inhibiting action.

#### DISCUSSION

The results show that a number of substances normally occurring in milk act as inhibitors. These substances—proteins, citrates, and lactose—are present in about the same amount in both summer and winter milk. They show a definite inhibiting action on washed cream. The proteins, particularly, as well as the citrates, to a smaller extent, inhibit by forming complexes with copper, thus removing the copper ions from the reaction. These complexes are formed by the free amino and carboxyl groups of the proteins similar to amino acids. Peterson and Walton (14) have found that various amino acids as well as uric acid inhibit the oxidation of ascorbic acid with copper by forming ions with copper. That it was a complex of copper was shown by the fact that the normal autoxidation of ascorbic acid was not inhibited by the amino acids in the absence of copper. They found that  $10^{-4}$  M cystine was the best inhibitor. Diehl (6) has included a theoretical discussion of copper complexes of amino acids and hydroxy acids through the coordinating groups so as to form chelate rings. These complexes prevented the ionization of copper.

Since proteins inhibit oxidized flavor by the formation of complexes with copper through the free amino and carboxyl groups, any proteolytic enzyme that splits the protein to give more free reactive groups would show a further inhibiting action. This is true of "Enzylac." That the inhibiting effect is not due to the preparation itself is shown by the fact that it does not inhibit oxidized flavor in washed cream.

The search for a special inhibitor present in milk from cows on pasture is complicated by the fact that the normal constituents of milk have an inhibitory action. Such a special inhibitor is entirely possible. However, the results showing that citrates, proteins, and lactose have an inhibitory effect on milk might indicate that the tendency to form oxidized flavor is balanced by these natural inhibitors. Although Brown and Olson (3) found that washed cream containing ascorbic acid and contaminated with copper gives oxidized flavor on pasture milk, the amount of oxidized flavor seemed to be less than that produced on winter milk. This suggests that the phospholipides in winter milk may be more unsaturated than in summer milk. One of the authors has started work on this consideration.

#### SUMMARY

A possible explanation for various inhibitors for oxidized flavor is presented. One class of substances which includes proteins, citrates, and amino acids inhibits by forming complex ions with copper, thus repressing its ionization.

Tyrosine, divalent manganese, lactose, and glucose inhibit oxidized flavor by being oxidized by hydrogen peroxide, thus protecting the phospholipides of milk.

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# THE EFFECT OF MACHINE MILKING UPON THE LEUCOCYTE COUNT AND THE CHLORIDE CONTENT OF MILK

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## INTRODUCTION

In an earlier paper (2) it was shown that the leucocyte count and the chloride content of milk are not reliable indices of udder infection. Individual differences between cows with respect to these two tests and differences in results caused by factors other than infection frequently are greater than the changes that occur when the udder becomes infected. Any factor that causes udder irritation doubtless will cause a rise in the leucocyte and chloride values.

The extent to which machine milking may influence leucocyte counts and chloride determinations, or other mastitis tests that reflect udder irritation, such as pH and catalase tests, is not clear. Munch-Petersen (4) reviewed the literature on mastitis and found opinion about equally divided with respect to milking machines as a factor contributing to mastitis. Dahlberg (1) found that leaving machines on the cows too long contributed to udder troubles and resulted in a drop in production early in the lactation period. He presented data indicating that reducing to 4 or 5 minutes the time interval that the machines were left on the cows was accompanied by a drop in the leucocyte count of the mixed herd milk. Hucker (3) reported that the leucocyte count of milk from machine-milked cows was higher than that of milk from hand-milked cows. In his machine-milked group there was a higher incidence of streptococcic infections than in his hand-milked group, a factor which probably exaggerated the difference he found between the two groups with respect to leucocyte counts.

In studies on the effects of milking machines on tests for mastitis there has not to the author's knowledge been any report which deals separately with infected and uninfected cows. If milking machines cause irritation which predisposes the udder to infection, one should expect detectable increases in the leucocyte count and chloride content of the milk when a cow is on machine milking as compared with values found for the same cow when she is on hand milking. On the other hand, if the machine is only a fomite spreading the infection without itself predisposing the udder to the infection, high leucocyte counts and chloride values would be found in the milk of machine-milked cows because of the high incidence of infection and not directly as a result of injury caused by the machines. The purpose of this

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paper is to report a study in which the effects of machine milking on cows are considered separately for infected and uninfected quarters.

#### METHODS

The data presented in this report were collected during the period from 1936 to 1942 in an investigation on mastitis in the Bureau of Dairy Industry herd at Beltsville, Maryland. The plan of the investigation was to begin with uninfected first calf heifers and continue them on the experiment as long as they remained in the herd. At intervals of 2 to 4 weeks individual quarter samples of the foremilk were taken from each cow and examined in the laboratory.

The examination consisted of the Hotis test, plating on Edwards aesculin crystal violet blood agar, a direct microscopic count of leucocytes and direct titration of chlorides with silver nitrate and a potassium chromate indicator. When there were abnormalities that could not be explained by the results on the Hotis test and by plating on the highly selective Edwards medium, the samples were plated in addition on standard tryptone glucose meat extract milk agar. Representative colonies were picked from the plates into meat infusion broth for identification of the organisms.

The herd at Beltsville is maintained primarily for experiments on feeding, breeding, and management. Changes from hand milking to machine milking or vice versa were made from time to time either in the interests of production records or for convenience in management. Usually when an animal was put on either hand or machine milking at the beginning of the lactation period, she remained on the same method of milking for the entire period. Occasionally, however, a change from one method of milking to the other was made during the lactation period. Under these circumstances it was possible to obtain data on 31 different cows that were on both methods of milking during the course of the investigation. For a few cows the data for a given method of milking extend over only parts of lactation periods, but for most of the cows one or more lactation periods were covered with each method of milking.

The data on the leucocyte counts and the chloride content for the different quarters have been divided into groups not only on the basis of the presence or absence of infection as determined by cultural tests, but also on the basis of the stage of lactation. For this purpose the lactation periods were divided into intervals of three months each. The data were also summarized for the entire lactation period.

#### THE EFFECT OF THE METHOD OF MILKING ON UNINFECTED QUARTERS

Table 1 shows the results obtained from the samples from the uninfected quarters of 27 cows. Of a total of 2,883 samples considered in this table, 1,571 were obtained while the cows were on hand milking and 1,312 while the cows were on machine milking.

*content of milk from uninfected quarters*

Cow No.	First 3 months of lactation					
	Hand*			Machine*		
	Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
	<i>number</i>	<i>per cent</i>	<i>thousands</i>	<i>number</i>	<i>per cent</i>	<i>thousands</i>
820	18	0.107	29	12	0.119	104
1235	6	0.128	82	15	0.186	804
1247	.....	.....	.....	16	0.108	36
1265	.....	.....	.....	20	0.099	26
1268	24	0.122	54	24	0.138	426
1269	13	0.110	287	35	0.113	269
1273	24	0.127	72	20	0.118	323
1274	20	0.111	31	14	0.102	235
1275	12	0.120	122	27	0.185	892
1278	16	0.124	52	10	0.111	310
1279	20	0.103	44	13	0.101	138
1282	32	0.105	24	24	0.130	488
1294	21	0.120	174	.....	.....	.....
1298	12	0.111	48	28	0.128	119
1418	.....	.....	.....	12	0.114	459
1434	33	0.120	77	.....	.....	.....
1441	.....	.....	.....	4	0.109	122
1445	16	0.094	26	47	0.090	188
1472	10	0.101	34	18	0.096	187
1473	10	0.088	22	32	0.110	917
1474	24	0.106	64	32	0.104	177
1476	16	0.116	159	19	0.104	1004
1478	.....	.....	.....	9	0.097	302
1483	12	0.110	116	.....	.....	.....
1488	15	0.089	23	21	0.099	372
1489	8	0.086	20	.....	.....	.....
1490	14	0.090	229	21	0.094	90
Total or average†	376	0.110	78	473	0.116	365

3 to 6 months in lacion						
820	15	0.117	20	15	0.132	257
1235	15	0.125	50	9	0.161	1051
1247	3	0.111	103	13	0.107	116
1265	.....	.....	.....	24	0.106	99
1268	40	0.116	94	.....	.....	.....
1269	15	0.102	200	28	0.117	186
1273	20	0.119	91	24	0.132	145
1274	16	0.114	75	28	0.106	170
1275	20	0.130	112	4	0.189	1402
1278	4	0.117	18	22	0.139	103
1279	.....	.....	.....	18	0.102	128
1282	40	0.113	25	16	0.143	605
1294	34	0.119	88	12	0.120	369
1298	.....	.....	.....	24	0.130	106
1418	.....	.....	.....	.....	.....	.....
1434	15	0.122	36	12	0.127	30
1441	.....	.....	.....	24	0.116	339
1445	22	0.105	44	37	0.095	45
1472	10	0.103	65	.....	.....	.....
1473	10	0.091	36	16	0.110	408
1474	20	0.116	306	36	0.119	637
1476	23	0.108	142	.....	.....	.....
1478	.....	.....	.....	22	0.101	151
1483	20	0.102	31	7	0.109	54
1488	16	0.096	23	8	0.091	169
1489	8	0.093	27	2	0.109	36
1490	.....	.....	.....	18	0.101	318
Total or average†	366	0.113	83	419	0.117	258

TABLE 1—(Continued)

Cow No.	6 to 9 months in lactation					
	Hand*			Machine*		
	Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
	<i>number</i>	<i>per cent</i>	<i>thousands</i>	<i>number</i>	<i>per cent</i>	<i>thousands</i>
820	12	0.126	54	9	0.186	383
1235	12	0.119	51	2	0.213	1505
1247	3	0.110	85	8	0.125	109
1265	14	0.134	190	4	0.121	45
1268	36	0.133	184	.....	.....	.....
1269	15	0.109	31	9	0.128	51
1273	20	0.137	149	16	0.162	351
1274	32	0.112	60	4	0.127	471
1275	20	0.161	346	.....	.....	.....
1278	.....	.....	.....	16	0.159	385
1279	.....	.....	.....	24	0.106	191
1282	40	0.119	20	4	0.240	2292
1294	6	0.111	76	8	0.136	439
1298	12	0.113	23	16	0.155	477
1418	.....	.....	.....	4	0.097	429
1434	23	0.142	156	26	0.139	116
1441	9	0.106	73	4	0.128	571
1445	14	0.112	92	19	0.124	159
1472	12	0.101	27	4	0.110	95
1473	10	0.105	388	8	0.112	365
1474	20	0.123	208	25	0.141	991
1476	30	0.107	116	.....	.....	.....
1478	.....	.....	.....	20	0.107	148
1483	16	0.107	69	12	0.124	165
1488	22	0.102	44	.....	.....	.....
1489	6	0.102	18	8	0.107	36
1490	.....	.....	.....	6	0.123	239
Total or average†	384	0.120	115	256	0.134	352

9 to 12 months in lactation						
820	21	0.139	409	6	0.167	317
1235	19	0.161	235	.....	.....	.....
1247	20	0.129	761	.....	.....	.....
1265	20	0.145	53	.....	.....	.....
1268	24	0.149	571	.....	.....	.....
1269	18	0.122	54	5	0.121	265
1273	24	0.165	165	11	0.270	4518
1274	38	0.113	47	.....	.....	.....
1275	12	0.190	676	.....	.....	.....
1278	.....	.....	.....	.....	.....	.....
1279	.....	.....	.....	16	0.141	809
1282	36	0.139	81	.....	.....	.....
1294	.....	.....	.....	.....	.....	.....
1298	24	0.119	22	.....	.....	.....
1418	16	0.099	61	3	0.108	103
1434	3	0.135	151	29	0.149	361
1441	.....	.....	.....	.....	.....	.....
1445	17	0.109	188	2	0.177	1748
1472	8	0.107	36	7	0.107	39
1473	10	0.104	45	5	0.095	40
1474	20	0.122	161	8	0.183	1280
1476	30	0.115	105	.....	.....	.....
1478	8	0.104	52	16	0.126	257
1483	12	0.119	104	12	0.130	218
1488	14	0.106	97	.....	.....	.....
1489	10	0.116	18	8	0.110	38
1490	16	0.100	20	.....	.....	.....
Total or average†	420	0.128	150	128	0.148	643

TABLE 1—(Continued)

Cow No.	More than 12 months in lactation					
	Hand*			Machine*		
	Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
	<i>number</i>	<i>per cent</i>	<i>thousands</i>	<i>number</i>	<i>per cent</i>	<i>thousands</i>
820	.....	.....	.....	15	0.178	1204
1235	.....	.....	.....	.....	.....	.....
1247	.....	.....	.....	8	0.177	859
1265	.....	.....	.....	.....	.....	.....
1268	.....	.....	.....	.....	.....	.....
1269	21	0.157	451	.....	.....	.....
1273	.....	.....	.....	.....	.....	.....
1274	.....	.....	.....	.....	.....	.....
1275	.....	.....	.....	.....	.....	.....
1278	.....	.....	.....	.....	.....	.....
1279	.....	.....	.....	.....	.....	.....
1282	.....	.....	.....	.....	.....	.....
1294	.....	.....	.....	.....	.....	.....
1298	.....	.....	.....	.....	.....	.....
1418	.....	.....	.....	.....	.....	.....
1434	.....	.....	.....	.....	.....	.....
1441	.....	.....	.....	.....	.....	.....
1445	.....	.....	.....	.....	.....	.....
1472	.....	.....	.....	9	0.142	328
1473	.....	.....	.....	.....	.....	.....
1474	.....	.....	.....	.....	.....	.....
1476	.....	.....	.....	.....	.....	.....
1478	.....	.....	.....	.....	.....	.....
1483	.....	.....	.....	.....	.....	.....
1488	.....	.....	.....	.....	.....	.....
1489	.....	.....	.....	.....	.....	.....
1490	4	0.098	18	4	0.104	435
Total or averaget	25	0.147	382	36	0.163	823
Summary for entire period						
820	66	0.123	153	57	0.153	500
1235	52	0.137	121	26	0.180	885
1247	26	0.125	80	45	0.122	218
1265	34	0.140	110	48	0.104	64
1268	124	0.129	205	24	0.138	426
1269	82	0.123	215	77	0.117	213
1273	88	0.138	119	71	0.156	700
1274	106	0.112	52	46	0.106	216
1275	64	0.148	293	31	0.186	958
1278	20	0.122	45	48	0.140	240
1279	20	0.103	44	71	0.112	305
1282	148	0.119	37	44	0.145	695
1294	61	0.119	117	20	0.126	397
1298	48	0.116	29	68	0.135	199
1418	16	0.099	61	19	0.110	397
1434	74	0.128	96	67	0.141	206
1441	9	0.106	73	32	0.117	341
1445	69	0.105	85	105	0.101	163
1472	40	0.103	40	38	0.110	184
1473	40	0.097	123	61	0.109	639
1474	84	0.116	179	101	0.125	630
1476	99	0.111	126	19	0.104	1004
1478	8	0.104	52	67	0.108	196
1483	60	0.108	73	31	0.123	160
1488	67	0.098	45	29	0.097	316
1489	32	0.100	21	18	0.108	37
1490	34	0.096	106	49	0.103	225
Total or averaget	1571	0.118	112	1312	0.124	368

The figures at the bottom of table 1, giving average results for all of the cows in this group, show that in every stage of lactation machine milking resulted in higher leucocyte counts and chloride values than did hand milk-

TABLE 2

*The effect of machine milking versus hand milking on the leucocyte count and chloride content of milk from infected quarters*

Cow No.	Infecting organism	First 3 months of lactation					
		Hand*			Machine*		
		Sam- ples	Average chlo- rides	Average leuco- cytes	Sam- ples	Average chlo- rides	Average leuco- cytes
		num- ber	per cent	thou- sands	num- ber	per cent	thou- sands
840	<i>Streptococcus</i>	15	0.168	2931	9	0.169	4331
859	<i>Streptococcus</i>	.....	.....	.....	8	0.175	4984
882	<i>Streptococcus</i>	12	0.182	5823	.....	.....	.....
1235	<i>Streptococcus</i>	5	0.150	5730	13	0.235	5083
1269	<i>Ps. aeruginosa</i>	7	0.118	3375	4	0.148	3822
1445	<i>Streptococcus</i>	11	0.114	2288	11	0.138	4290
1472	<i>Streptococcus</i>	10	0.121	573	6	0.116	1692
1473	<i>Ps. aeruginosa</i>	10	0.122	1230	11	0.178	9355
1479	<i>Ps. aeruginosa</i>	8	0.093	1860	.....	.....	.....
Total or average†		78	0.137	2874	62	0.173	5169
		3 to 6 months in lactation					
840	<i>Streptococcus</i>	6	0.175	1901	21	0.171	2895
859	<i>Streptococcus</i>	2	0.211	5140	14	0.180	5668
882	<i>Streptococcus</i>	6	0.176	1702	6	0.199	8157
1235	<i>Streptococcus</i>	5	0.135	141	19	0.180	4889
1269	<i>Ps. aeruginosa</i>	5	0.113	5022	2	0.159	17500
1445	<i>Streptococcus</i>	15	0.121	940	9	0.158	2310
1472	<i>Streptococcus</i>	10	0.125	370	20	0.120	2524
1473	<i>Ps. aeruginosa</i>	10	0.133	6337	23	0.170	10329
1479	<i>Ps. aeruginosa</i>	7	0.101	785	2	0.133	2975
Total or average†		66	0.134	2188	116	0.164	5446
		6 to 9 months in lactation					
840	<i>Streptococcus</i>	3	0.177	1129	12	0.209	5723
859	<i>Streptococcus</i>	13	0.127	1031	3	0.207	3273
882	<i>Streptococcus</i>	15	0.142	2054	.....	.....	.....
1235	<i>Streptococcus</i>	4	0.118	77	6	0.245	6407
1269	<i>Ps. aeruginosa</i>	5	0.108	801	5	0.153	8150
1445	<i>Streptococcus</i>	8	0.139	562	6	0.215	2521
1472	<i>Streptococcus</i>	12	0.137	1065	16	0.127	3519
1473	<i>Ps. aeruginosa</i>	10	0.166	7370	18	0.135	12346
1479	<i>Ps. aeruginosa</i>	6	0.112	2625	8	0.141	2754
Total or average†		76	0.144	2142	74	0.166	6397

\* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.



TABLE 2—(Continued)

Cow No.	Infecting organism	More than 9 months in lactation					
		Hand*			Machine*		
		Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
		number	per cent	thousands	number	per cent	thousands
840	<i>Streptococcus</i>	.....	.....	.....	3	0.181	2276
859	<i>Streptococcus</i>	6	0.166	1215	.....	.....	.....
882	<i>Streptococcus</i>	6	0.207	2701	.....	.....	.....
1235	<i>Streptococcus</i>	6	0.157	321	.....	.....	.....
1269	<i>Ps. aeruginosa</i>	13	0.164	9548	17	0.145	4511
1445	<i>Streptococcus</i>	4	0.176	6417	.....	.....	.....
1472	<i>Streptococcus</i>	18	0.146	1219	18	0.120	1479
1473	<i>Ps. aeruginosa</i>	20	0.144	6850	.....	.....	.....
1479	<i>Ps. aeruginosa</i>	6	0.153	8531	8	0.180	6692
Total or average†		79	0.158	4877	46	0.144	3558
Summary for entire period							
840	<i>Streptococcus</i>	24	0.171	2448	45	0.181	3895
859	<i>Streptococcus</i>	21	0.170	1671	25	0.181	5162
882	<i>Streptococcus</i>	39	0.169	3259	6	0.199	8157
1235	<i>Streptococcus</i>	20	0.142	1577	38	0.209	5195
1269	<i>Ps. aeruginosa</i>	30	0.136	5896	28	0.148	5990
1445	<i>Streptococcus</i>	38	0.129	1827	26	0.163	3196
1472	<i>Streptococcus</i>	50	0.134	883	60	0.121	2392
1473	<i>Ps. aeruginosa</i>	50	0.142	5727	52	0.160	10821
1479	<i>Ps. aeruginosa</i>	27	0.113	3234	18	0.157	4529
Total or average†		299	0.144	3066	298	0.163	5333

\* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

ing. With some exceptions the results for each individual cow also show higher leucocyte and chloride levels when milking machines were used. With cow 1,269 the differences are so small as to be insignificant. On the other hand, certain cows, such as 1,235 and 1,275, show much higher results on machine milking than on hand milking. The results on cows such as those two tend to exaggerate the group average figures for machine milking.

Summary figures on cow 1,265, shown in the section on the extreme right of table 1, indicate that the leucocytes and chlorides in the milk were lower while she was on machine milking than they were while she was on hand milking. The figures for the different stages of lactation show, however, that for 1,265 all of the results on machine milking were obtained during the first six months of lactation and all of the results on hand milking were obtained during the last six months of lactation. That lack of direct comparison possibly accounts for the apparently lower results with machine

milking. This surmise is borne out by the group average figures at the bottom of table 1, which show that with both methods of milking there is a steady increase in leucocytes and chlorides as lactation advances.

#### THE EFFECT OF THE METHOD OF MILKING ON INFECTED QUARTERS

Table 2 shows the results for the infected quarters of 9 cows that were on both hand and machine milking after the onset of infection. Of a total of 597 samples reported here, 299 were taken while the cows were on hand milking and 298 while they were on machine milking.

As would be expected, the leucocyte counts and chloride values in these samples were distinctly higher than those reported in table 1 for the samples from uninfected quarters. The variations found in table 2 are greater than those found in table 1 and there is less tendency with these infected quarters for the leucocytes and chlorides to rise with advancing lactation.

In general, the results in table 2 indicate that with infected quarters, as with uninfected quarters, the leucocyte counts and chloride values are higher when the cows are on machine milking than when the cows are on hand milking.

#### RELATION OF THE METHOD OF MILKING TO THE OCCURRENCE OF INFECTION

Whenever a newly infected quarter was found during this investigation an attempt was made to correlate the onset of the infection with the method of milking that was being used at the time. Infections that occurred during the dry period and a few infections that occurred at about the time a cow was changed from one method of milking to the other were not ascribed to either method of milking.

Of 23 streptococcic infections, the onset of which could be correlated with the method of milking, 9 occurred in cows on hand milking and 14 in cows on machine milking. During the period that these infections occurred, the numbers of cows on the two methods of milking were approximately the same. Since the total number of traceable cases was small, it is doubtful whether the difference between the 9 cases occurring on hand milking and the 14 cases occurring on machine milking is significant.

On the other hand, infections with *Pseudomonas aeruginosa* are rather definitely associated with machine milking. The onset in 29 cases of *Ps. aeruginosa* can definitely be traced to periods when the affected cows were on machine milking. Only one case has been found that appears to have originated when the cow was on hand milking. The evidence with respect to several doubtful cases is that they, also, probably are associated with machine milking.

Infections associated with organisms other than streptococci and *Ps. aeruginosa* were too few to be of significance in this study.

## DISCUSSION

At the time this investigation was under way little attention was given to the length of time that the machines were left operating on the cow. The general practice was to remove the teat cups as soon as milk flow ceased, but when it was known that a cow was not letting her milk down, the machine was left on the cow for an indefinitely longer period in an effort to accomplish complete milking. According to Petersen (5) this practice will ultimately make the cow a stripper. Petersen further asserts that to leave the machine on the cow after the milk has ceased flowing may injure the lining of the teat cistern.

Dahlberg (1) has shown that cows on machine milking remain in high production longer and give milk with fewer leucocytes if the machines are left on the cow a maximum of 4 or 5 minutes at a milking. Some of the manufacturers of milking machines are now recommending an interval of milking time not to exceed 5 minutes, insisting that slow milking cows can be trained to fast milking with benefit to both production and the health of the udder.

It is quite probable that if, during this investigation, the practice now recommended for limiting milking time to 5 minutes had been adhered to, the difference in results between hand and machine milking would have been less than was actually found. Just how much less is purely conjecture. It is apparent that under the conditions of this experiment the use of milking machines caused some udder irritation and contributed significantly to the spread of *Ps. aeruginosa* infections. The relationship between the use of milking machines and the incidence of streptococcic infections in the herd is not definite.

## SUMMARY AND CONCLUSIONS

The effect of milking machines on cows has been studied by comparing the results of leucocyte counts and chloride titrations of the milk when the cows were on machine milking with the results found when the same cows were on hand milking. Data for infected and uninfected quarters were studied separately. A total of 31 cows was included in the two groups.

Under the conditions of this experiment the milk of most of the cows when on machine milking yielded higher leucocyte and chloride values than when they were on hand milking. This was true for both infected and uninfected quarters. There were marked differences between cows in their response to machine milking.

Milking machines apparently contributed to the spread of *Ps. aeruginosa* infections in the herd. Whether they were a factor in the spread of streptococcic infections is not definite.

The influence of the interval of time that the machines were left on the cows as a factor in causing the high leucocyte and chloride levels with machine milking is discussed.

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## AMMONIATED SUGAR BEET PULP AS A NEW NITROGENOUS FEED FOR RUMINANTS

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It has been shown (9, 10) that sugar beet pulp can be easily ammoniated to produce a palatable product containing 4 to 5 per cent nitrogen. The added nitrogen is stable and mostly in a water-soluble form. Studies (11) on pilot plant ammoniation of the dried pulp indicate that commercial ammoniation would be a relatively simple and inexpensive process. Since considerable evidence (3, 4, 5, 13) has accumulated to show that ruminants can use urea and ammonium bicarbonate for their maintenance and growth it seemed desirable to learn whether ruminants can also use ammoniated pulp for their nitrogenous nutrition. The experimental work reported in this paper was designed to give information on that point.

The term ammoniated pulp as used in this paper refers to a plain dried sugar beet pulp which has been treated with ammonia (11) to raise its nitrogen content. Likewise, ammoniated molasses pulp refers to a molasses beet pulp which has been treated with ammonia to increase its nitrogen content. Molasses pulp is a product usually made by mixing molasses with wet beet pulp at the beginning of the drying process.

On May 12, seven Holstein male calves weighing 125 to 187 pounds were placed on a milk and calf meal diet. These animals developed scours soon thereafter which held them back somewhat. They were fed milk, calf meal, alfalfa and molasses beet pulp from May 12 to July 26 with the animals receiving little milk the last month.

At the beginning of the experimental period the animals were placed in separate specially prepared pens inside an enclosed hay barn. Each pen was further protected during the coldest part of the winter and liberal amounts of pine shavings were used as bedding. Even so, it was very cold in these pens during the subzero weather. The animals were allowed to run in a vegetation-free paddock when the weather would permit.

The ammoniated pulps were prepared by adding 300 pounds of pulp to the closed ammoniation unit. Fifteen pounds of ammonia were added and the unit was revolved for thirty minutes. The temperature became 130° C. and a product containing 25–27 per cent protein ( $N \times 6.25$ ) was obtained.

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Unammoniated pulp was then mixed with the ammoniated to give the necessary protein value for the rations.

On July 26, the animals were placed on the experimental rations shown in table 1 and each animal was referred to by the ration he ate. The rations were essentially alike except for the nitrogen source and the fact that starch was substituted for nitrogen free molasses in some cases.

TABLE 1  
*The composition of the basal ration*

Ingredients	lbs.	Protein	
		%	lbs.
Plain dried beet pulp .....	60.00	9.2	5.52
Timothy hay .....	16.00	8.0	1.28
Starch .....	8.79	0.0	0.00
Molasses .....	12.00	0.0	0.00
Steamed bone meal .....	2.00	7.0	0.14
Iodized salt .....	1.00	0.0	0.00
Fortified fish oils .....	0.21	0.0	0.00
	100.00	6.94	6.94

Calves 1 and 2 received the basal ration. This contained 6.94 protein ( $N \times 6.25$ ). Rations 1 and 2.

Calf 3 received the basal ration modified by substituting ammoniated pulp for plain pulp and starch for molasses. Total protein ( $N \times 6.25$ ) was 12.42 per cent. Ration 3.

Calf 4 received the basal ration modified by substituting ammoniated pulp for plain pulp. Total protein ( $N \times 6.25$ ) was 12.42 per cent. Ration 4.

Calf 5 received the basal ration modified by substituting ammoniated pulp for plain pulp and starch for molasses. Total protein ( $N \times 6.25$ ) was 17.02 per cent. Ration 5.

Calf 6 received the basal ration modified by substituting ammoniated pulp for plain pulp. The protein ( $N \times 6.25$ ) was 17.02 per cent. Ration 6.

Calf 7 received the basal ration except toasted soybean meal was substituted for the starch and molasses. Total protein ( $N \times 6.25$ ) was 16.42 per cent. Ration 7.

Calf 8 received the basal ration except molasses ammoniated pulp was substituted for plain pulp. Total protein ( $N \times 6.25$ ) was 17.02 per cent. Ration 8.

Molasses was excluded from rations 3 and 5 to note whether this soluble carbohydrate promoted the use of the nitrogen in the ammoniated pulp.

The calves were fed equal amounts of feed three times daily and sufficient fortified fish oils were added to each feeding to supply each animal daily with 2700 units of vitamin A and 500 units of vitamin D per 100 pounds of body weight. Individual weights were taken weekly

during the experimental periods and the increase was recorded as growth. The animals were weighed daily the last three days and the mean was taken as the final value.

Pictures were taken of the animals 132 and 222 days from the beginning of the experiment. Calves 1 and 2 were taken from the basal diet and placed on rations 8 and 5 respectively, 150 days from the beginning of the experiment. The second pictures were taken 72 days after the protein deficient animals had been changed to diets containing ammoniated pulp.

Blood was taken from the jugular vein of each animal 97, 140 and 210 days after the beginning of the experiment. It was analyzed for total erythrocytes, hemoglobin, total leucocytes, neutrophiles, eosinophiles, monocytes and lymphocytes by the usual clinical methods. The color index was then calculated by the usual procedure. The amounts of glucose, calcium, total serum protein, non-protein nitrogen, urea, cholesterol, serum albumin and serum globulin were determined (6, 7) and the albumin-globulin ratio was calculated.

The steers were slaughtered at the Swift and Company yards under federal inspection and the liver from No. 7 and a kidney from each animal were sent to the pathological laboratory for further investigation.

The animal tissues were analyzed (1) for total protein, water-soluble protein, coagulable protein and moisture. The A.O.A.C. method was modified for the water-soluble and coagulable-proteins on the livers and kidneys as follows:

Ten to thirteen grams of freshly ground sample was exhausted with 10 cc. of water at room temperature. The sample was transferred to a 250-cc. volumetric flask with water and diluted almost to the mark and stored in the refrigerator 65 hours at 2° C. Samples were removed from the refrigerator in pairs and made up to volume immediately. They were mixed well and the entire 250 cc. was centrifuged thirty minutes. The supernatant portion was decanted and 25-cc. room-temperature aliquots were used for water-soluble proteins and 75-cc. for coagulable proteins. One cc. of 0.1 N acetic acid was added to the 75 cc. and the sample was boiled five minutes to coagulate the proteins. The coagulum was transferred to a filter and washed with 250 cc. of hot water for the livers and 25 cc. hot water for the kidneys. Nitrogen was determined by the Kjeldahl method and protein was calculated ( $N \times 6.25$ ).

#### EXPERIMENTAL RESULTS

It was not necessary to work the animals on to these feeds for the palatability was such that they ate well from the beginning except for the first animal on ration 6. He did not clean his feed up readily until he was placed upon ration 4 later in the experiment. Each animal consumed 5 pounds of feed early in the experiment. This was increased from time to

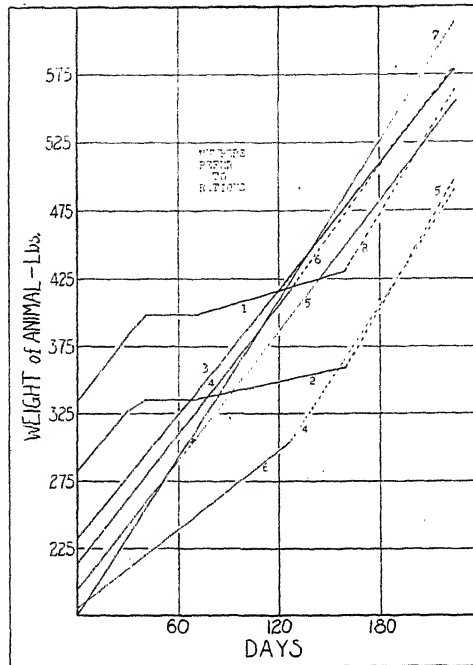


FIG. 1. Growth of male calves—225 days.

time until after 34 days 12 pounds were being eaten. After 70 days the amount was increased to 15 pounds. This proved to be too much so the amount was decreased to 11 pounds for the rest of the experiment. The

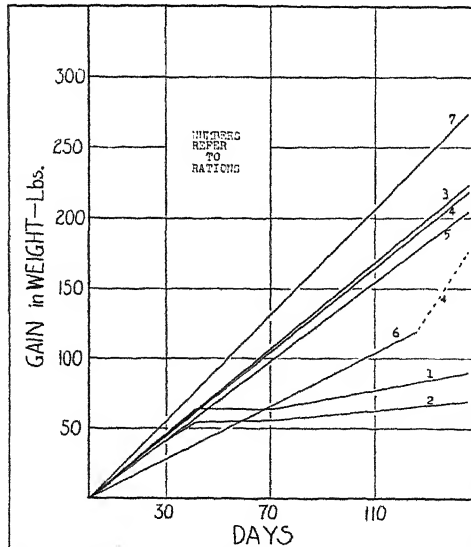


FIG. 2. Growth of male calves—146 days.



appetites were good and equalized feeding was practiced throughout without difficulty. No digestion troubles of any kind developed. There was no evidence of diuresis in any of the animals. The growth of the animals is shown in figures 1, 2 and 3. Figure 1 shows the weight of the animals during the experiment (225 days). Figure 2 shows the gain in weight after 146 days and figure 3 shows the gain in weight at the end of 225 days.

The animals on rations 1 and 2 continued to grow quite rapidly during the first 40 days of the ration but began to develop protein deficiency symptoms

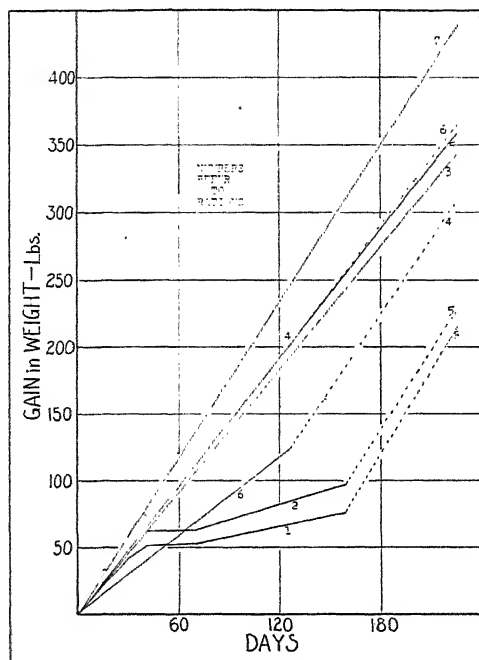


FIG. 3. Growth of male calves—225 days.

at the end of 30 days. Then they did not grow at all for 30 days, after which they grew very slowly until they were placed on rations 8 and 5, respectively.

The protein deficiency symptoms as shown in figure 4 became very marked before they were placed on the rations containing ammoniated pulp. The animals became very thin, developed a sharp-boned hump in their back, a pot belly, a rough and shaggy coat and a listless gait.

After the protein-deficient animals were placed on rations 8 and 5 they started to lose their protuberant bellies, their backbones straightened out, their coats took on more life, they became much more lively, and began to grow at the rate of about 2 pounds per day.

There was little difference in the growth rates of animals 3, 4 and 5. Molasses was excluded from rations 3 and 5 to note whether the absence of

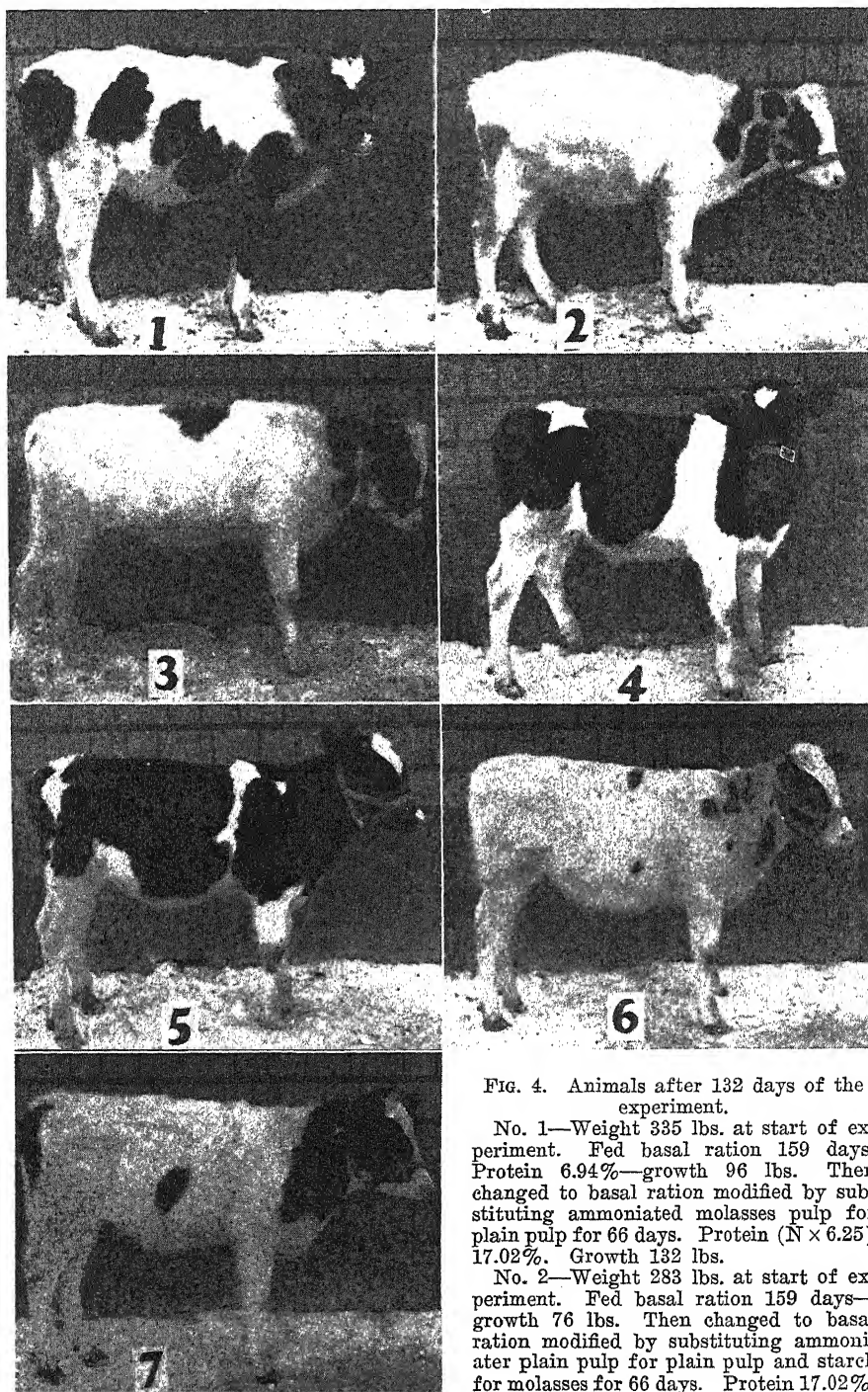


FIG. 4. Animals after 132 days of the experiment.

No. 1—Weight 335 lbs. at start of experiment. Fed basal ration 159 days. Protein 6.94%—growth 96 lbs. Then changed to basal ration modified by substituting ammoniated molasses pulp for plain pulp for 66 days. Protein ( $N \times 6.25$ ) 17.02%. Growth 132 lbs.

No. 2—Weight 283 lbs. at start of experiment. Fed basal ration 159 days—growth 76 lbs. Then changed to basal ration modified by substituting ammoniated plain pulp for plain pulp and starch for molasses for 66 days. Protein 17.02%. Growth 138 lbs.

this source of soluble carbohydrate inhibited the use of the nitrogen in the ammoniated pulp or conversely whether its presence promoted nitrogen utilization over that of starch alone. The results indicate no difference in the growth of the animals on rations containing all starch as compared to those in which molasses replaced part of the starch.

The animal on ration 6 grew very slowly during the preliminary 14 weeks before the experiment was started. Furthermore, he continued to grow more slowly than animals 3, 4, 5 and 7 from the beginning of the experiment. This animal was on the ration containing molasses and pulp ammoniated to 4.16 per cent nitrogen. He did not eat it as well as the other animals were eating their rations. Therefore, after 159 days the rations of animals 4 and 6 were exchanged to learn if there was something about ration 6 to give the inferior results. After this the slow growing animal grew much faster on ration 4 while the new animal on ration 6 continued to eat and grow at its previous rate on ration 4. Apparently it was not the ration alone but probably an animal-ration relationship causing the poor initial results from ration 6. Growth was just as good on rations containing 12.42 per cent protein as on those containing 17.02 per cent protein.

The pictures of the animals at the end of the experiment are shown in figure 5. They show that the animals on ammoniated pulp grew during the 90-day interval between pictures and that the physical condition of the protein deficient animals was greatly improved by the ammoniated pulp.

The data in table 2 show the daily gains and feed consumed per 100 pounds gain in weight for the various growing periods shown in figure 1. The soybean fed animal gained 1.956 pounds per day for 225 days. Ration one provided only 14.26 per cent of this growth for animal 1; ration two, 10.38 per cent; ration three, 78.07 per cent; ration four, 81.80 and 95.55 per cent; ration five, 81.34 and 106.90 per cent and ration six, 49.89 and 84.15 per cent. The larger value on ration 5 was obtained when it was fed to animal 2 that had been on the protein deficient diet. Animal 4 did about equally well on rations 4 or 6 while animal 6 grew much better on ration 4 than ration 6. The feed consumed per 100-pound gain as compared to those listed by Morrison (14) must be considered good.

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No. 3—Initial weight 234 lbs. Fed basal ration modified by substituting ammoniated plain pulp for plain pulp and starch for molasses. Protein 12.42%. Growth after 159 days 244 lbs.; after 225 days 343 lbs.

No. 4—Initial weight 214 lbs. Fed basal ration modified by substituting ammoniated plain pulp for plain pulp. Protein 12.42%. Growth after 159 days 261 lbs.; after 225 days 364 lbs.

No. 5—Initial weight 195 lbs. Fed basal ration modified by substituting ammoniated plain pulp for plain pulp and starch for molasses. Protein 17.02%. Growth after 159 days 255 lbs.; after 225 days 353 lbs.

No. 6—Initial weight 181 lbs. Fed basal ration modified by substituting ammoniated pulp for plain pulp for 126 days. Protein 17.02%. Growth 123 lbs. Then changed to ration four for 99 days. Growth 185 lbs.

No. 7—Initial weight 174 lbs. Fed basal ration modified by substituting toasted soybean meal for starch. Protein 16.42%. Growth after 159 days 313 lbs.; after 225 days 440 lbs.

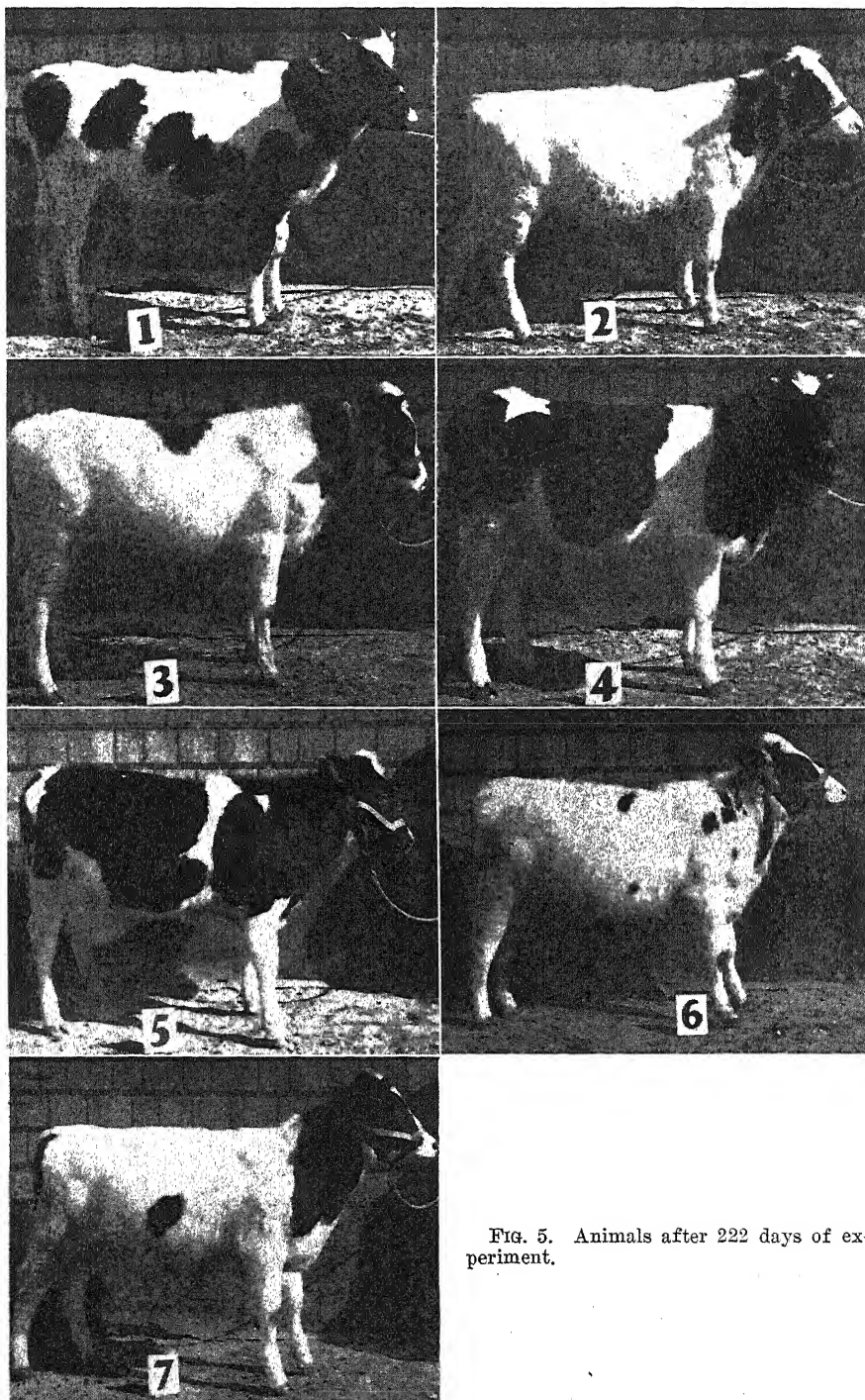


FIG. 5. Animals after 222 days of experiment.

## ANALYSES OF THE BLOOD

The data on the blood are shown in table 3. The first two blood samplings were taken while animals 1 and 2 were still on the deficient rations. The third samples were taken near the end of the experiment and after animals 1 and 2 had been changed to the ammoniated rations and had gained rapidly in weight. Thus, blood from animals on ammoniated pulp was obtained from the third sampling of animals 1 and 2 and from all three samplings from animals 3, 4, 5 and 6.

The blood from animal No. 7 represents that from an animal on a conventional protein source. The approximate normal value (2) for cattle

TABLE 2

*Rate of gain and amount of feed consumed by steers during certain feeding periods*

Steer No.	Ration No.	Experimental period	Daily gain	Portion of daily gain promoted by soybean meal	Total feed consumed	Feed consumed per 100 lbs. body weight
		<i>days</i>	<i>lbs.</i>	<i>%</i>	<i>lbs.</i>	<i>lbs.</i>
1	Basal	41-159	0.279	14.26	1463	4433
	Ration 8	159-225	2.000	102.25	726	550
2	Basal	41-159	0.203	10.38	1463	6096
	Ration 5	159-225	2.091	106.90	726	526
3	Ration 3	1-225	1.527	78.07	2545	742
4	Ration 4	1-126	1.600	81.80	1456	728
	Ration 6	126-225	1.646	84.15	1089	667
5	Ration 5	1-225	1.591	81.34	2545	711
6	Ration 6	1-126	0.976	49.89	1456	1184
	Ration 4	126-225	1.869	95.55	1089	589
7	Ration 7 (Soybean meal)	1-225	1.956	100.00	2545	579

listed in table 3 represents mean values and are largely based on adult cattle. Miller (12) has shown that the number of erythrocytes decreases in calves as they mature while the hemoglobin values remain quite constant with wide differences in individual animals. He shows that calves appear to have more leucocytes than the adult with the proportions of the kinds of leucocytes remaining about the same as in adults. He also shows that young bovine animals have less serum protein than adults. Hodgson *et al.* (8) show that the glucose in the blood of dairy animals expressed in milligrams per 100 cc. of blood was 100.4, 88.2, 80.2, 75.4, 69.6, 67.8, 62.2 and 55.0 after 6 days, 4 weeks, 3, 7, 11, 15, 19 and 23 months, respectively.

The data show the total erythrocytes to be normal in all cases. The third sample from animal No. 7 was somewhat higher than the other values. The hemoglobin was normal in all samplings and the total leucocytes were about the same except that the first two samplings on the soybean-meal animal were high. Perhaps the abnormal leucocyte and erythrocyte values on the soy-

TABLE 3  
Composition of blood of growing Holstein steers

Steer No.	Ration No.	Blood sampling date	Erythrocytes		Color index	Leucocytes					
			Total count per cu. mm.	Hemo-globin		Total count per cu. mm.	Neutro-philles (adults)	Eosino-philles	Mono-cytes	Lympho-cytes	
				<i>gms./100 cc.</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	1	1 *	7,450,000	9.7	0.42	12,700	14	4	.....	82	
	1	2	8,560,000	9.8	0.37	12,000	29	1	.....	70	
	8	3	7,930,000	9.7	0.40	8,500	23	.....	.....	77	
2	2	1	7,550,000	9.7	0.40	6,600	26	1	1	72	
	2	2	9,240,000	9.7	0.34	6,000	18	1	.....	81	
	5	3	7,810,000	9.2	0.40	9,300	11	1	2	86	
3	3	1	8,850,000	12.2	0.45	8,800	27	.....	1	73	
	3	2	7,410,000	10.7	0.47	11,050	24	4	1	70	
	3	3	7,600,000	9.2	0.40	10,700	29	.....	.....	70	
4	4	1	9,630,000	10.6	0.36	8,700	34	1	1	64	
	6	2	8,480,000	9.2	0.36	7,800	27	.....	.....	73	
	6	3	8,700,000	10.7	0.40	12,200	27	.....	.....	73	
5	5	1	9,600,000	10.3	0.35	4,800	23	1	2	64	
	5	2	9,190,000	10.7	0.35	7,200	24	2	.....	74	
	5	3	8,840,000	10.7	0.39	7,900	28	.....	.....	72	
6	6	1	9,650,000	11.2	0.40	6,300	27	2	.....	71	
	4	2	9,370,000	9.8	0.34	8,250	22	1	1	76	
	4	3	9,100,000	10.6	0.37	13,700	31	.....	.....	69	
7	7	1	7,500,000	9.4	0.40	23,900	25	.....	1	74	
	7	2	8,630,000	9.9	0.32	22,200	27	3	.....	70	
	7	3	11,560,000	11.7	0.33	10,100	46	.....	.....	54	
Approximate normal for cattle (2)			6,325,000	10.3	.....	7,900†	21	5	10	64	

TABLE 3—(Continued)

Steer No.	Ration No.	Blood sampling date	Glucose	Calcium	Cholesterol	Non-protein nitrogen	Urea N	Total serum protein	Serum		Albumin/globulin ratio
									Albumin	Globulin	
			mgms./100 cc. blood	mgms./100 cc. serum	mgms./100 cc. serum	mgms./100 cc. blood	mgms./100 cc. blood	per cent			
1	1	1*	74.0	11.51	88	18.0	11.0	6.56	3.94	2.62	1.50/1
	1	2	69.5	9.70	127	26.9	9.2	5.60	3.51	2.09	1.68/1
	8	3	83.0	10.00	81	32.0	15.4	4.38	2.48	1.90	1.31/1
2	2	1	73.0	12.93	58	16.0	7.8	7.88	4.28	3.60	1.19/1
	2	2	74.5	10.70	121	.....	7.1	5.66	3.51	2.15	1.63/1
	5	3	94.0	10.60	62	33.5	11.2	5.05	3.00	2.05	1.46/1
3	3	1	45.0	12.73	44	26.0	9.2	7.98	4.52	3.46	1.31/1
	3	2	82.0	10.90	101	34.1	9.3	7.49	3.85	1.64	2.34/1
	3	3	89.0	10.50	69	32.0	8.1	5.22	3.55	1.67	2.13/1
4	4	1	77.0	12.42	55	24.8	9.0	5.49	3.94	1.55	2.54/1
	6	2	77.5	11.10	85	33.3	10.0	4.76	3.32	1.44	2.31/1
	6	3	97.0	11.10	71	29.5	9.1	6.42	3.42	3.00	1.14/1
5	5	1	65.0	11.31	58	30.0	16.3	6.18	4.10	2.08	1.97/1
	5	2	81.0	10.60	81	26.5	15.1	5.45	4.15	1.30	3.19/1
	5	3	89.0	9.60	72	32.0	16.0	5.33	3.45	1.88	1.84/1
6	6	1	55.0	11.92	66	32.0	14.6	6.49	4.09	2.40	1.70/1
	4	2	75.0	10.90	61	36.6	11.3	4.83	3.36	1.47	2.29/1
	4	3	86.0	10.10	75	32.0	15.1	5.15	3.64	1.51	2.41/1
7	7	1	73.0	11.92	70	30.4	14.4	6.67	4.28	2.39	1.79/1
	7	2	78.5	11.10	118	29.8	15.1	5.62	3.47	2.15	1.61/1
	7	3	92.0	11.10	106	34.5	17.0	5.67	.....	.....	.....
Approximate normal for cattle (2)			40-60	9-12	50-230	20-40	6-27	4-8.5	7.2	.....	.....

\* 1—Oct. 30, 1942.

2—Dec. 12, 1942.

3—Feb. 20, 1943.

† 5,000-12,000 (12).

bean-meal-fed animal were associated with the diffuse cirrhotic liver found in the animal when slaughtered. The adult neutrophiles were much the same in all cases except in the last blood samples from the soybean-fed animal.

The lymphocytes were much the same in all animals.

The glucose values were similar in all animals and while slightly above that listed as normal for the adult they must be considered normal for animals of this age. Feed was never withheld before taking the blood samples, for Hodgson (*loc. cit.*) shows that this has no effect on the blood sugar of the bovine. Carbohydrate metabolism appeared to be functioning normally.

The calcium content of the blood fell between the normal ranges in all samplings and was remarkably constant. All the cholesterol values but one fell within what is listed as normal for cattle.

The non-protein and urea nitrogen of the blood was low during the period the animals were on the protein-deficient diet. The third sampling, which was taken after these animals were placed on diets containing more nitrogen, showed an increase in non-protein nitrogen and urea. All animals were metabolizing nitrogen in a normal manner. It is interesting to note that in case of the animals on ration 3 and 4 the urea values were less than one-third of the non-protein nitrogen values while the urea values for the blood of the animals on ration 5, 6 and 7 are almost half those of the non-protein values. The non-protein values are much the same whether the ration was 12.4 or 17.02 per cent protein while the urea values were about 1.5 times larger for the rations having the higher protein values.

Total serum protein was normal and much the same in all samplings. The serum albumin and globulin values for the animals on the ammoniated pulp diets were similar to those of the control animal and confirm the work of Miller (12) that serum protein is lower in young animals than in adults. The albumin-globulin ratios were similar in all cases.

The animals were slaughtered at Swift and Company under federal supervision. All animals and internal organs were pronounced normal by the federal inspectors except the liver in animal No. 7. This liver showed a diffuse cirrhosis. Macroscopic examinations of one kidney from each animal showed each to be a normal healthy kidney.

The carcasses were held in the coolers for 48 hours after the animals were slaughtered. A rib steak was then taken from each animal and tested for color and flavor at the Swift and Company Laboratories. The meat from each animal was found to be normal in color and flavor. Section of the ribs were taken from each animal and roasted and found to be normal in flavor, color and odor.

Table 4 shows the weight of the animals before they went into the coolers and the dressing percentage. The animals were not in a good grade of finish for the experiment was not designed for this purpose. However, a very small amount of fat was deposited on the inside of the ribs and they brought



TABLE 4

*The weight of the animals when dressed and before going into the coolers*

Animal No.	Dressed wt. before going into cooler	Dressing percentage*
	<i>lbs.</i>	
3	300	52.00
4	299	51.73
5	278	50.27
6	254	51.94
7	339	55.21

\* Based on feed lot wt.

\$10.25 per hundred weight. The soybean animal had a slightly higher dressing percentage than the animal fed ammoniated pulp.

Part of the liver, one kidney and five ribs were taken from each carcass and analyzed for the constituents shown in table 5. Since the cuts from the different steers contained different amounts of fat some variation in the analyses from animal to animal is expected.

The results on the ribs, livers and kidneys show that the animals fed ammoniated pulp contained much the same amount of fat, total protein, water-soluble protein, coagulable protein and moisture as the soybean-fed animal. This fact, together with the growth increments, is conclusive evidence that the animals were using the ammonia added to the dried beet pulp for their protein metabolism.

TABLE 5

*Composition of tissue (moisture free basis)*

Animal number	Rib cut				
	Fat	Total protein	H <sub>2</sub> O sol. protein	Coagulable protein	Moisture
	%	%	%	%	%
3	31.7	65.3	14.8	7.1	70.3
4	19.5	73.4	16.2	9.3	72.2
5	23.3	74.9	17.0	7.8	72.9
6	13.9	83.1	19.7	9.6	75.1
7	21.3	75.1	17.5	9.0	73.1
	Livers				
	Fat	Total protein	H <sub>2</sub> O sol. protein	Coagulable protein	Moisture
	%	%	%	%	%
3	7.1	69.0	27.8	12.0	71.6
4	5.6	72.0	26.2	11.5	71.4
5	4.1	67.8	30.0	15.2	71.0
6	5.6	67.8	26.5	11.3	71.7
7	6.7	73.1	26.2	15.0	69.9
	Kidneys				
	Fat	Total protein	H <sub>2</sub> O sol. protein	Coagulable protein	Moisture
	%	%	%	%	%
3	30.3	60.3	29.0	9.5	73.8
4	19.7	70.3	32.6	14.6	76.1
5	35.1	58.7	25.6	9.2	70.8
6	32.7	56.5	28.3	7.9	73.5
7	38.7	49.5	26.0	6.9	71.1

## DISCUSSION OF RESULTS

Animals on the basal diet grew very little while those on the basal ration plus ammoniated pulp for 225 days grew as much as 81.34 per cent of the growth promoted by the toasted soybean meal. Animals on a low protein diet and then changed to a diet in which the nitrogen was raised only by the ammoniated pulp grew 102 and 107 per cent of that of the soybean meal animal. Ration 6 gave only about half as good results as soybean meal with one animal but gave about 84 per cent of soybean meal when fed to another animal. The animal which did so poorly on ration 6 grew 95.55 per cent as well as the soybean meal animal when placed on ration 4. The animals on the ammoniated diet grew at the rate of 1.5, 1.65, 1.59 and 1.87 pounds per day while those animals changed from the basal ration to a ration containing ammoniated pulp grew at the rate of 2.0 pounds per day. The

TABLE 6

*Basal rations of three non-protein nitrogen experiments for growing Holstein calves*

	After Hart <i>et al.</i> (3)		Present experiment
	Holstein males	Holstein females	Holstein males
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
Yellow corn .....	29.5	20.0	.....
Dried beet pulp (plain) .....	.....	.....	60.0
Ground timothy .....	47.0	47.5	16.0
Starch .....	20.0	24.0	20.79
Corn molasses .....	.....	10.0	.....
Steamed bone meal .....	2.0	2.0	2.0
Salt .....	1.0	1.0	1.0
Cod liver oil .....	0.5	0.5	0.21
Total .....	100.0	105.0	100.0

soybean-meal-fed animal grew at the rate of 1.96 pounds per day. It is interesting to compare these results with those of the two experiments reported by Hart *et al.*, since Holstein calves were used in all three experiments and the diets were very similar except that sugar beet pulp was used in place of yellow corn and the amount of timothy varied somewhat. This comparison is shown in tables 6 and 7.

The animals on urea and ammonium bicarbonate grew at the rate of 0.8 to 1 pound per day while those on ammoniated pulp for 225 days grew at the rate of 1.53 to 1.64 pounds per day.

It has been shown (13) that ruminants are able to use non-protein nitrogen compounds because the microflora in their stomachs convert such nitrogen into bacterial protoplasm protein.

The enzymes pepsin, trypsin and peptidases then act on the proteins to liberate amino acids in the intestines where they are absorbed into the blood stream.



Rose *et al.* (15) have shown that the amino acids lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine are indispensable for the rat and dog. Since the animals on ammoniated pulp did not grow as well as the one on soybean meal it would appear that either all of the essential amino acids were not synthesized or not synthesized in sufficient quantity to give maximum growth. This leads to the interesting question of whether the growth promoting ability of ammoniated pulp for ruminants would be increased when fed in practical diets containing normal and multiple supplementary nitrogen sources such as barley, corn and alfalfa hay with these materials supplying less natural protein than is needed by the animal for maximum growth. Under such conditions if the growth were not maximum the question of supplying in an economical form those essential amino acids not synthesized by the ruminants, should be an interesting and fruitful study.

#### SUMMARY

1. An experiment on the ability of Holstein calves to grow on ammoniated plain sugar beet pulp has been conducted.
2. The experiments indicate that the animals can use such nitrogen sources for their nutritional needs. They grew at the rate of about 1.6 pounds per day as compared with 1.96 pounds for one animal on toasted soybean meal.
3. Animals fed a diet in which starch was substituted for molasses grew as well as those on rations containing the molasses. This indicated that the soluble carbohydrate did not further the use of the nitrogen by the microorganisms in the digestive tract any better than starch.
4. The animals grew just as well on rations 12.42 per cent protein as those on 17.02 per cent protein ( $N \times 6.25$ ).
5. No diuresis of any of the animals occurred.
6. The blood was analyzed for fifteen different constituents three times during the experiment and found to be normal.
7. The animals and their internal organs passed federal inspection at the Swift and Company yards. The soybean-meal animal had a diffuse cirrhotic liver. This is not evidence that the liver condition was connected with the soybean meal. Macroscopic examinations of the kidneys showed them to be normal.
8. Rib and liver cuts as well as one kidney from each animal were analyzed and found to be normal in protein. The color and flavor of the meat was normal.
9. Further work is indicated.

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ANNOUNCEMENT  
THIRTY-NINTH ANNUAL MEETING

JUNE 20-22, 1944

CALL FOR PAPERS

May we ask all members of the association who contemplate presenting papers for the consideration of the program committee to send the titles to the chairman, Professor H. P. Davis, Dairy Husbandry Department, University of Nebraska, Lincoln 1, Nebraska, at once. Titles will be accepted until April 1, but it is desired that they be received as early as possible because the program committee cannot meet and will have to carry on its work by correspondence. Unless otherwise arranged for by the committee, all papers are not to exceed 12 minutes in length and abstracts must be in the hands of the committee chairman not later than June 1.

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## LIVE WEIGHT AND MILK-ENERGY YIELD IN BRITISH GOATS

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The London Dairy Show, staged normally in mid-October each year, embraces a milch goat competition based on milk and fat yields for a two-day period. Records of these competitions are published in the Journal of the British Dairy Farmers' Association and include live weights of the individual goats at time of the contest, as well as various data of milk yield.

The present article deals with these two-day records (on a per-day basis) for the 17 years, 1920-1937 except 1927, and is particularly concerned with the size-yield relationship.

### STAGE OF LACTATION

Since goats have a pronounced tendency to bear their young in the spring season they are usually rather far advanced in stage of lactation at time of the contest in October. For present purposes all records more than 360 days after kidding are rejected (14 records, 486 to 959 days after kidding). All other records are used, a total of 318 distributed as follows, with respect to 30-day month after kidding:

Month .....	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th	11th	12th
Number .....	1	5	5	14	28	38	85	98	40	1	2	1

Apparently kidding occurs most frequently in the month of March. The delayed stage of lactation must be kept in mind in considering the magnitude of yields. Assuming a linear lactation curve, the daily yield for the first 8 months of lactation would be somewhat greater than the daily yield for the contest period. Of course, what the yields represent is only the contest period, for the goats selected to enter the contest. Naturally a goat with too little persistency, that is, milking at too low a level at the time would not be entered.

### AVERAGE PERFORMANCE BY BREEDS

Table 1 shows the average of the 318 records to be 8.36 pounds of milk and 0.390 pounds of fat per day at an average stage of lactation 195 days

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<sup>1</sup> On leave of absence for military service.

TABLE 1

*Average data (daily basis) for goats by breeds, in order of live weight, W, at the London Dairy Show*

Breed	n	Stage ds.	Fat %, raw	Fat %, weighted	Milk	Fat	FCM	W	100FCM/W*
					lbs.	lbs.	lbs.	lbs.	lbs.
Toggenburg .....	22	185	4.08	4.09	7.33	0.300	7.44	130	5.71
Unidentified .....	106	185	4.86	4.76	6.51	0.309	7.25	134	5.46
Anglo Nubian Swiss .....	25	209	4.77	4.69	8.86	0.416	9.78	153	6.43
British Toggenburg .....	24	192	4.57	4.54	8.76	0.398	9.47	155	6.13
Saanen .....	17	213	4.72	4.66	8.48	0.395	9.31	163	5.80
Anglo Nubian .....	18	177	6.37	6.30	7.08	0.446	9.52	168	5.73
British Alpine .....	45	197	4.61	4.55	9.31	0.423	10.08	173	5.97
British .....	20	211	4.74	4.65	10.72	0.499	11.77	176	6.69
British Saanen .....	41	213	4.46	4.42	11.51	0.509	12.24	176	7.04
All breeds .....	318	195	4.76	4.66	8.36	0.390	9.19	154	5.99

\* Averages of the individual 100FCM/W values.

after kidding. The average milk-energy yield, FCM, is 9.19 pounds of 4 per cent milk.<sup>2</sup> The average of the 318 fat percentages is 4.76 but when weighted by milk yield this becomes 4.66.

As between breeds considerable variation is shown. The breed class designated "unidentified" is largely composed of "just goats" but includes a few animals properly belonging in another class, where the breed identity could not be ascertained from the published record. The breeds are arranged in table 1 in order by average live weight. The larger size and greater productivity of the British strains (British Alpine, British, British Saanen) are conspicuous.

TABLE 2

*Variance with respect to certain items, as between and as within certain groups—London Dairy Show goats. Live weight, W, in pounds; milk and FCM in pounds per day*

Group	Item	Degrees of freedom		Variance		F	5% F	1% F
		Between	Within	Between	Within			
Breed	Live weight	8	309	13,289.0	643.0	20.67	1.97	2.57
"	Milk	"	"	123.1	4.4	27.78	"	"
"	FCM	"	"	128.9	5.5	23.37	"	"
"	100FCM/W	"	"	11.7	2.3	5.07	"	"
Age	Live weight	9	308	3,225.0	896.0	3.60	1.91	2.48
"	FCM	"	"	10.6	8.6	1.24	"	"
"	100FCM/W	"	"	5.2	2.5	2.08	"	"
Live weight	FCM	16	301	64.2	5.7	11.31	1.68	2.07
"	100FCM/W	"	"	3.1	2.5	1.24	"	"

<sup>2</sup> FCM =  $0.4 \times \text{milk} + 15 \times \text{fat}$ . One pound FCM = 340 kilocalories milk energy = 0.034 pounds milk protein regardless of the fat percentage of the natural milk. This relation is known to hold quite accurately in cows and is presumed to be applicable in goats.



## LIVE WEIGHT AND YIELD

The variance data of table 2 show that the breeds are distinctly different with respect to live weight and FCM yield; less distinctly (although significantly in the statistical sense) with respect to FCM/W, that is, milk energy per unit live weight. Reference to table 1, column headed 100FCM/W, indicates the breed differences in FCM/W arise in a tendency for the larger breeds to produce more milk energy per unit live weight than the smaller breeds. Evidently the British strains are being developed for large size and milking proclivity; and milking capacity appears to be fully proportional to the larger size, as between breeds.

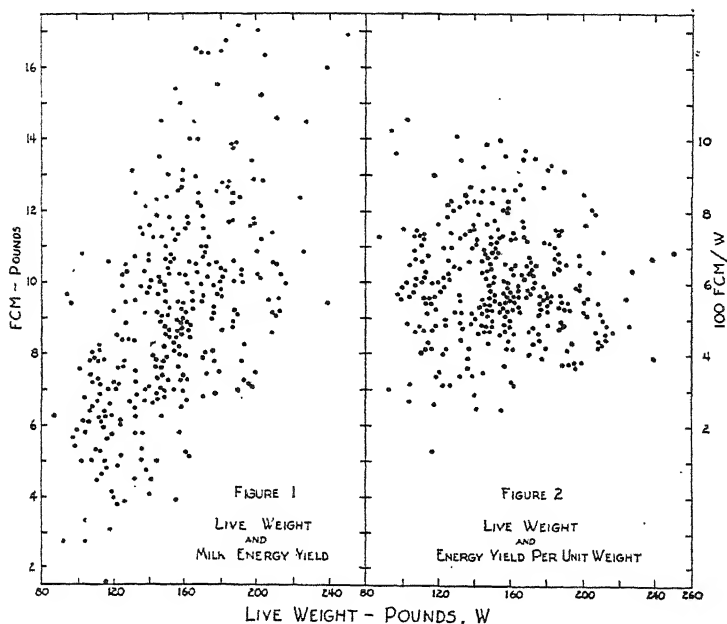


FIG. 1, FCM, in pounds per day plotted against live weight in pounds for each of the 318 records.

FIG. 2, FCM, in pounds per day per 100 pounds live weight plotted against live weight in pounds, same records as figure 1.

Figure 1 shows each of the 318 FCM records plotted against live weight without regard to breed. FCM ranges from 1.5 to 17.2 pounds per day and live weight ranges from 88 to 250 pounds. There is a fairly close correlation between live weight and milk-energy yield, amounting to  $r=0.57$ . If milk-energy yield is expressed as a power function of live weight,  $FCM=aW^b$ , it is found that  $b=0.91$ . From table 1 if FCM is expressed as  $FCM=aW^b$  it comes out that  $b=1.54$ , as between breeds; and  $b=0.57$  as within breed.

Figure 2 shows each of the 318 FCM/W records plotted against live weight. If FCM is proportional to  $W^{0.91}$  (figure 1) it should follow that FCM/W is proportional to  $W^{-0.09}$ , that is FCM/W decreases slightly with increasing W. Table 2 shows, however, that FCM/W variance as between live weight groups differs only slightly from the variance within live weight groups. In other words FCM/W appears to be independent of W in the 318 records taken as a whole.

#### DISCUSSION

There are some elements of uncertainty as to the broad meaning of the present size-yield data. The goats entered in the contest are a selected lot, to meet the competition which is predominantly one of points for production, viz.: 1 point per pound of milk plus 20 points per pound of fat plus 4 points per pound of solids-not-fat. No distinction is made as to size of animal except indirectly by distinguishing between first-kidders and older<sup>3</sup> animals. Since a 100-pound goat competes on a par with a 200-pound goat in terms of absolute yield it follows that selection must be more severe in the smaller size animals. This tends to bias FCM/W, as concerns an unselected population, making it unduly large for small goats and unduly small for large goats. Just how far this may affect the data of figure 2 (and figure 1) cannot be said.

What is needed is records on an unselected population for the first 8 months of lactation with live weight measured within 31 days after kidding. In the present records variation in the stage of lactation affects not only the daily yield for the 2-day period but affects also the numerical value of  $b$  in the regression,  $FCM = aW^b$ , at least such is true in cows. For example, in Holstein cows, dealing with 8-months FCM and live weight measured at 10 stages of lactation, viz., within the first month (31 days) after calving, within the second month, etc.,  $b$  is affected as follows:

Month	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th
$b =$	1.01	0.96	0.93	0.94	0.92	0.89	0.91	0.82	0.82	0.83

For the present goat data the average stage of lactation is within the 7th month after kidding and  $b = 0.91$  which agrees with the 7th month in the Holstein data. By inference if live weight in the goats had been measured within 31 days after kidding we might expect to find  $b = 1.01$ .

Finally it may be noted that the average of the 318 records is 5.99 pounds FCM per day per 100 pounds live weight, 60 pounds per 1000 pounds live weight. This is perhaps nearly double what we should find in cows under comparable conditions. Metabolic tempo (metabolism per cell

<sup>3</sup> In table 2 it will be noted there is no significant difference between age classes with respect to either FCM or FCM/W. For these records age distinctions are wholly without importance, but live weight distinctions are very important.

per unit time) as between goats and cows follows a " $\frac{3}{4}$  power rule" with respect to live weight; but within species metabolic tempo is independent of live weight.

#### SUMMARY AND CONCLUSIONS

This article deals with 318 records of goats in two-day milking competition at the London Dairy show. The milk-energy yield, FCM, ranges from 1.5 to 17.2 pounds per day. Live weight, W, ranges from 88 to 250 pounds. The correlation between W and FCM is 0.57. In the power equation regression,  $FCM = aW^b$ ,  $b = 0.91$ .

Attempting to bring the data into line with FCM for the first 8 months of lactation and live weight measured within 31 days after kidding it appears probable that b in the regression  $FCM = aW^b$  may be safely taken at unity. That is, milk-energy yield in well-developed milch goats tends to be proportional to live weight.



# FURTHER STUDIES ON OXIDATION OF VITAMIN A AND CAROTENE IN MILK FAT

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Our previous study (2) of the effect of storage temperature upon the rates of destruction of vitamin A and carotene in milk fat protected from the light but exposed to the air has indicated that following an induction period, simultaneous progressive destruction of vitamin A and carotene occurs in samples held at 40°, 50°, and 60° C. Since that time additional data have been obtained concerning the changes in vitamin A and carotene contents of the milk fat held at lower temperatures than its melting point; namely, the region within which under favorable conditions the fractional crystallization of glycerides takes place.

We also reported that the reconstitution test is very useful in recognizing the flavor defects of the milk fat and thus the extent of oxidative deterioration. This was evident from the fact that the milk made of pasteurized skimmilk and post-induction period fat developed off flavors, which continued to increase rapidly in their intensities instantaneously after processing, while the one containing fresh fat showed fairly good keeping quality. Subsequently it was observed that the latter milk at the end of 24 hours' storage at 5° C. developed off flavor, although of much lower intensity than the other but nevertheless identified by judges as slightly oily or tallowy and which lowered its score from 23 to 21 points, as compared with the score of zero for the first milk at the end of the same period.

Since the conditions for oxidation appear to be favorable, when fat is redispersed in skimmilk, we can assume that the above technique can be of value in readily detecting changes in the resistance of milk fat to oxidation, whether these changes are brought about during storage, by exposure to light, or any other factor.

For these studies the vitamin A and carotene contents of the fat were determined by the Koehn and Sherman method (3), while in the preparation of milk fat we followed the same procedure as described in the former paper (2).

## EXPERIMENTAL

### *The Effect of Rapid Solidification of Milk Fat Upon the Rates of Destruction of Vitamin A and Carotene*

In a study of the relationship between the temperature of storage and the rates of destruction of vitamin A and carotene in the milk fat (2), it was

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pointed out that the samples of fat in the "liquid" state were placed in incubators at constant temperatures. At the end of the holding period at temperatures below the melting point, these samples were found to be well separated into solid crystalline and liquid fractions. It is apparent, therefore, that the system involved was quite different from the one represented by fat stored at higher temperatures.

The additional data concerning this particular experiment are presented in figure 1. They show that at higher temperatures vitamin A and carotene are destroyed at approximately the same rate, whereas at lower temperatures vitamin A is destroyed more rapidly.

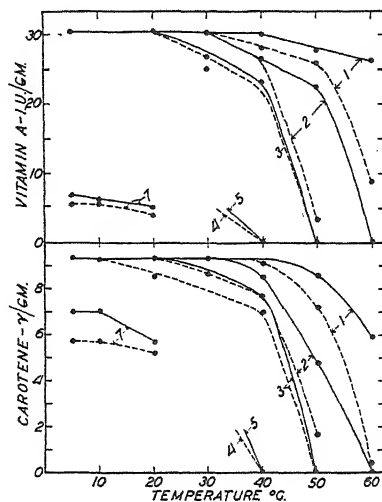


FIG. 1. The relation between the temperature of storage and the rates of destruction of vitamin A and carotene in the fat from (1) milk (solid line) in which lipolysis was checked by pasteurization immediately after excretion by mammary gland, and (2) milk (dotted line) in which lipolysis was accelerated. The fat was exposed to the air but protected from the light. Numbers 1-7 on the graphs indicate months of storage.

There are no significant differences in the rates of destruction between the samples held at 5°, 10°, and 20° C., or between the fats isolated from normal and rancid milks.

In order to learn if the physical state of the fat at the time of storage was a factor governing the rates of destruction of vitamin A and carotene, 25 grams of fat were weighed into half-pint milk bottles, to provide a sufficient surface contact between the fat and outside air. Several of these samples were held first at -14° C. for a period of time to insure a rapid solidification of the fat and then stored at 5° and 20° C. The remaining samples while still in a liquid state were placed at the same temperatures.

The data are presented in table 1. It is apparent that rapid precooling of fat retards the destruction of vitamin A and carotene during the storage at both 5° and 20° C.

TABLE 1

*The effect of solidification of milk fat upon its vitamin A and carotene*

Fat from normal pasteurized milk

Physical state previous to storage	Stored at temperature	Vitamin A	Carotene
	$^{\circ}\text{C.}$	$\text{I.U./gm.}$	$\gamma/\text{gm.}$
S.	5	21.1	4.3
L.	5	13.6	3.5
S.	20	18.4	4.2
L.	20	11.0	3.8

The samples were analyzed at the end of seven months' storage in the dark. Symbols indicate: S.—solid, and L.—liquid, fats, respectively.

The physical states of these samples at the end of storage were quite different. The samples which were cooled rapidly at  $-14^{\circ}\text{C.}$  remained firm, retaining their homogeneous fine texture; the others had a coarse spongy semi-solid texture. Since atmospheric oxygen plays an important part in oxidative deterioration of milk fat protected from the light, it is possible to assume that the destruction of vitamin A and carotene might be traced in part to the rates of diffusion of atmospheric oxygen into the samples.

The keeping quality of different solid fats might vary with their degree of hardness, a factor determining the rate of crystallization.

These results suggest that a functional relationship exists between the

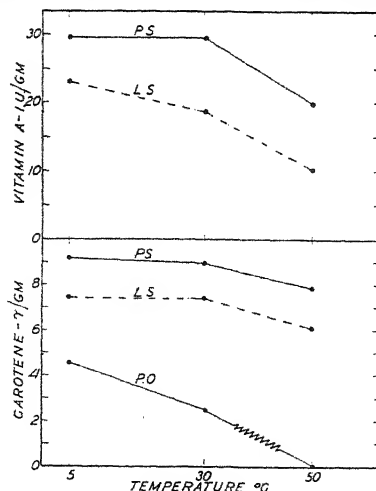


FIG. 2. The vitamin A and carotene contents of samples of milk fat in tightly sealed (S.) or open (O.), plain (P.) and lacquered (L.) tin cans after seven months' storage at indicated temperatures. In plain open tin can (P.O.) the sample was colorless at the end of two and one-half months of storage at  $50^{\circ}\text{C.}$  In the open lacquered cans the samples were all colorless at the end of seven months of storage.

rates of influx of atmospheric oxygen on one hand and the rates of destruction of vitamin A and carotene on the other.

In order to test this possibility, samples of fat in tightly sealed or open tin cans, both lacquered<sup>1</sup> and plain, were placed while still in the liquid state at temperatures of approximately 5°, 30°, and 50° C. These cans were filled to the top. The vitamin A and carotene contents of the tightly sealed samples were determined after storing for 7 months, while carotene only was determined in the other samples.

The data presented in figure 2 show that in the absence of free surface contact with atmospheric oxygen the destruction of vitamin A and carotene proceeds at a much slower rate or not at all, depending upon the temperature of storage and the type of container used.

It seems probable, therefore, that in absence of light, only when fat is exposed to atmospheric oxygen is its physical state a factor governing the rates of destruction of vitamin A and carotene. Subsequently, it was found that the induction period of fat could be considerably prolonged by covering the exposed surface with a sheet of tin foil to exclude the air.

#### *The Destruction of Vitamin A and Carotene Resulting from Reemulsification of Milk Fat in Pasteurized Skimmilk*

Preliminary work has been done to show the extent to which vitamin A and carotene of the milk fat is reduced by reemulsification in skimmilk and some of the factors involved in this phenomenon.

In a previous paper (2) it was shown that at the point when the vitamin A content of fat exposed to light generated by a mercury vapor lamp was reduced to a minimum, the concentration of its precursor remained practically unchanged. The data in figure 3 show that at the end of 30 minutes of exposure the vitamin A drops from a value of 33 I.U./gm. to 4.5 I.U./gm. Irradiation for an additional 90 minutes causes no further drop in vitamin A. It did not seem reasonable to us that part of the vitamin A would resist destruction, so it may be assumed that the value of 4.5 I.U./gm. is due to an artifact. That this is so, is supported by the observation that no absorption peak in the 620 m $\mu$  region of the spectrum could be detected with the Beckmann spectrophotometer following the Carr-Price reaction, nor could an absorption peak be found in the 326 m $\mu$  region with the unsaponifiable fraction dissolved in diethyl ether.

It was found (2) that atmospheric oxygen plays an important part in the photochemical destruction of carotene, while in the case of vitamin A an additional photochemical reaction caused its rapid destruction. Therefore, it seemed of interest to learn if the exposure of fat to light, prior to its reemulsification in pasteurized skimmilk, renders carotene more susceptible to oxidation.

<sup>1</sup> Sanitary enamel.



For this reason the irradiation experiment was repeated following the same procedure as previously described, with the exception that the temperature of fat was maintained at 50° C. and the intensity of the mercury vapor lamp at 19,000 foot candles.<sup>2</sup> The samples were irradiated for various periods of time up to 120 minutes. At the end of each period they were divided into two parts. One part was immediately analyzed for vitamin A and carotene, while the other one was reemulsified<sup>3</sup> in pasteurized skimmilk, then held for 24 hours before separation and analysis.

The data are presented in figure 3. Although the carotene content of the samples was not altered by irradiation, its susceptibility to oxidation in the

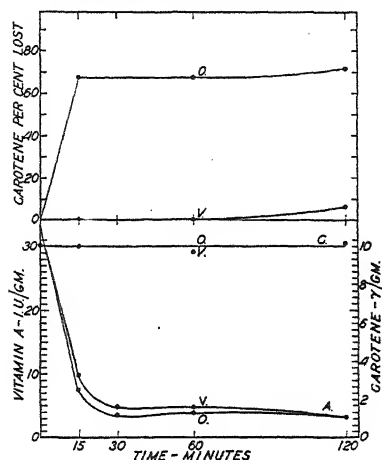


FIG. 3. The effects of irradiation and of reemulsification of irradiated milk fat in pasteurized skimmilk upon vitamin A and carotene. The effect of irradiation alone is shown in the lower chart. The upper chart shows the losses in carotene (per cent) due to reemulsification of fat. Symbols on the graphs indicate: C—carotene, A—vitamin A, O. and V.—fat irradiated in open or under vacuum sealed tubes.

presence of skimmilk was definitely affected when the fat was irradiated in open tubes. In this case 70 per cent of the carotene was lost due to the reemulsification of fat in pasteurized skimmilk, whereas the fat which was irradiated in vacuum sealed tubes was not affected.

Subsequently it was thought worth while to obtain some idea concerning the destruction of vitamin A and carotene produced by irradiation, such as might be encountered under ordinary conditions.

Samples of fat in 18-mm. test tubes filled to the top and left open were irradiated for 10 hours with northern daylight passing through window glass.<sup>4</sup> (This sample had been stored for 4 months at 5° C. in the dark.)

<sup>2</sup> Weston light meter.

<sup>3</sup> In Club aluminum cream maker.

<sup>4</sup> The per cent transmission of incident light (Beckmann spectrophotometer) of this glass was as follows: 360 mμ—75.3%; 340 mμ—42.0%; 330 mμ—16.5%; 325 mμ—7.0%; 320 mμ—2.0%; 310 mμ—0%.

TABLE 2

*The effect of irradiation with daylight upon vitamin A and carotene of milk fat*  
Milk fat

Irradiated with daylight		Reemulsif. in pasteur. skimmilk	Vitamin A	Carotene
<i>Hrs.</i>	<i>In</i>		<i>I.U./gm.</i>	$\gamma$ / <i>gm.</i>
U.	.....	Before	19.4	9.6
U.	.....	After	11.0	9.2
10	O.T.	Before	6.0	8.3
10	O.T.	After	5.1	1.8
U.	.....	Before	29.5	9.0
U.	.....	After	12.3	7.8
8	P.D.	Before	12.3	7.4

O.T.—open tube, P.D.—Petri dish, U.—unirradiated control.

The light intensity throughout the duration of exposure varied from 200 to 400 foot candles as measured by a Weston light meter. Immediately after, a portion of this fat and one of the control samples were reemulsified separately in pasteurized skimmilk to produce a reconstituted milk containing 5 per cent fat. They were reseparated after holding for 24 hours at 5° C. These samples and the remaining portions were then analyzed for vitamin A and carotene. In another experiment a sample of fat in a covered Petri dish filled to the top was exposed for 8 hours only. (This sample had been stored for 7 months at 5° C. in a tightly sealed plain tin can.)

The data are presented in table 2. These results are in substantial agreement with the preceding observations.

A study of absorption spectra and chromatograms of carotene from irradiated and non-irradiated milk fat samples indicate that no change in the carotene occurred. The irradiation was carried out as previously described in 18-mm. test tubes by northern daylight. This indicates that the effect of irradiation is upon constituents of the fat other than carotene.

Finally the data of table 3 show the effect of irradiation with northern daylight upon vitamin A and carotene of fresh milk fat. To part of the

TABLE 3

*The effect of irradiation with daylight upon vitamin A and carotene of fresh milk fat*  
Milk fat

Irradiated with daylight		Reemulsif. in pasteur. skimmilk	Vitamin A		Carotene
			Original	Original and added	
<i>Hrs.</i>	<i>In</i>		<i>I.U./gm.</i>	<i>I.U./gm.</i>	$\gamma$ / <i>gm.</i>
U.	.....	Before	22.1	250.0	5.1
U.	.....	After	19.3	246.0	4.7
8	O.T.	Before	21.7	221.0	5.0
8	O.T.	After	17.0	163.0	3.9

O.T.—open tube, U.—unirradiated control.

sample crystalline vitamin A was added so that it contained 250 I.U. of vitamin A per gram of fat. Both samples of fat in 18-mm. test tubes were irradiated for 8 hours. The light intensity throughout the duration of exposure was less than 200 foot candles.

The results of this experiment were practically the same as the others, except that the losses in vitamin A produced by reemulsification of fresh unexposed fat in pasteurized skimmilk were rather small.

These observations appeared to be of a practical importance since they show that considerable losses in vitamin A, accompanied by the development of oily flavor, occur in the fat exposed to light, thus affecting not only the nutritive value of the product but its palatability as well.

Finally it should be noted that the destruction of vitamin A in the milk fat was accompanied by the development of oily flavor, whereas that of carotene was accompanied by the development of tallowy flavor (1, 2).

The data suggest that a relationship exists between the ability of fat to resist oxidation and the stability of carotene. It is possible that a partial or complete destruction of antioxidant renders fat more susceptible to oxidation, thus indirectly affecting carotene.

#### CONCLUSIONS

The present study shows that the resistance of both vitamin A and carotene to oxidation by redispersing the fat in pasteurized skimmilk decreases upon exposure of fat to light or after its prolonged storage in the dark.

Milk fat can be stored for several months even at 60° C. without loss of vitamin A or carotene providing the fat is degasified then placed in light-proof containers filled to the top and tightly sealed.

Rapid precooling as compared with slow cooling of milk fat retards the destruction of vitamin A and carotene during its storage at both 5° and 20° C. in open-to-the-air but protected-from-the-light containers.

The data indicate that milk fat containing free fatty acids shows more rapid loss of vitamin A and carotene during its storage at 40°-50° C. in open-to-the-air but protected-from-the-light containers.

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## ZINC IN COWS' MILK\*

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### INTRODUCTION

References in the literature to the zinc content of cows' milk are rather infrequent. Several investigators report its presence in the spectrographic analysis of milk ash (3, 6, 7, 11, and 12), but only three report quantitative determination of the amount present (2, 4, and 8). Information on the influence of the amount of zinc in the feed on the zinc content of milk is practically negligible. Wright and Papish (11) in 1929 found more than the usual amount of zinc in milk obtained from cows pasturing in the neighborhood of a zinc smelter. Broek and Wolff (5) in 1935 found no marked variations in this element that could be attributed to feeding practice.

As part of a comprehensive project on the minerals of cows' milk, the effect of feeding zinc oxide on the zinc content of milk was investigated at this station during the past winter (1942-43).

### EXPERIMENTAL

The procedure was similar to that followed in earlier work on manganese (1). Eight cows were divided into two groups of four each, with an Ayrshire, a Guernsey, a Holstein, and a milking Shorthorn in each group. The breed pairs were matched as closely as possible with regard to stage of lactation, none of the cows being beyond the 10th week in lactation when the work was begun. One group received the supplement during November, December and January; the other group received it during February, March and April. The amount of zinc oxide fed was ten grams daily mixed with the grain allowance. No ill effects were evident as a result of feeding this amount of zinc. Except for the feeding of the supplement the rations and management of the two groups were in all respects identical.

Composite two-day milk samples of one liter each were taken from each cow once a month. Zinc was determined in triplicate 200 ml. portions of each sample by the turbidity method of Bodansky (4) as quoted by Scott (10). As some slight modifications were introduced, the detailed procedure is given below.

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<sup>1</sup> Acknowledgment is made to Vernon C. Cole, who did the analytical work under the author's direction.

*Method for Determination of Zinc in Milk*

Solutions required: *Standard zinc solution.* Dissolve 0.1 gram of zinc (C.P.) in 10 ml. of HCl (sp. gr. 1.20); Dilute to one liter. 1 ml. = 0.0001 gram of zinc.

*Citric acid solution*—50%

*Sulphuric acid*—1 in 3.

*Ammonium thiocyanate solution*—2%

*Hydrochloric acid*—1 in 5.

*Potassium ferrocyanide solution*—2%

*Procedure.* Measure out 200 ml. of milk into a glazed silica evaporating dish and evaporate to crispness on a steam bath. Ash in an electric muffle furnace at just below perceptible redness; the dishes are placed in the cold furnace and the temperature is raised gradually to avoid frothing and spattering. The ash should be white or with not more than a tinge of gray. Cool and dissolve the ash with 25 ml. conc. HCl and 10 ml. conc.  $\text{HNO}_3$ ; boil moderately on a hot plate for half an hour. Add 10 ml. of  $\text{H}_2\text{SO}_4$  (1 in 3) and evaporate to dryness on a steam bath. Dissolve the residue in water and adjust the acidity to contain about 5 per cent  $\text{H}_2\text{SO}_4$  (probable volume 150 cc.) and precipitate the heavy metals by bubbling  $\text{H}_2\text{S}$  through the liquid for an hour.

Filter off the heavy metals and boil the filtrate on a hot plate to remove  $\text{H}_2\text{S}$ ; cool, add two drops of methyl red, neutralize with  $\text{NH}_4\text{OH}$  and add 10 ml. of a 50 per cent citric acid solution. Heat to boiling on a hot plate (watch out for bumping at this point) and if no calcium citrate separates add small quantities of calcium carbonate at a time until a precipitate of about one gram of calcium citrate is formed. Remove from heat and while still very hot pass a stream of  $\text{H}_2\text{S}$  through the solution until cool and for four hours thereafter. Filter through a small paper and wash with a 2 per cent solution of ammonium thiocyanate. Dissolve the precipitate in 25 ml. of HCl (1 in 5) heated almost to boiling, wash the paper with water, cool the filtrate and make up to 50 ml.

Place a 25-ml. aliquot of the filtrate in a 50-ml. Nessler tube and hold until a series of standards of appropriate range has been prepared by measuring portions of the standard zinc solution into other 50-ml. Nessler tubes. Dilute the standard and unknown solutions to about 40 ml. and add 3 ml. of HCl (sp. gr. 1.20) to the standards, and 2 ml. of 2 per cent potassium ferrocyanide solution to all the tubes. Dilute the contents of each tube to the mark and mix thoroughly. After two or three minutes compare the turbidity of standards and unknown in a suitable comparator rack with a white tile base and illuminated by fluorescent light. Calculate the percentage of zinc from the quantity of sample taken and the standard solution similar in turbidity to that of the sample. Standards used in this work were 2, 3, 4, 5, 6, 7, and 8 ml. of the standard zinc solution per 50 ml. of final volume. The method is sufficiently sensitive to permit matching to one-half ml. of the standard (0.00005 gram of Zn).

TABLE 1  
Effect on zinc content of the milk of feeding cows zinc oxide (winter of 1942-43). Milligrams of zinc per liter of milk

Month	Cows receiving supplemental Zn					Cows on control ration				
	1st half of the season									
	A291	G626	II539	S38	Average all four	A320	G632*	II539	S62	Average all four
November	3.8	7.2	5.7	5.7	5.6	3.4	5.3	3.4	1.9	3.5
December	6.3	7.2	4.3	6.7	6.1	5.8	3.9	4.3	5.3	4.8
January	4.3	6.7	4.3	3.8	4.8	3.4	4.3	3.4	2.8	3.5
Average 1st half	.....	.....	.....	.....	5.5	.....	.....	.....	.....	3.9
2nd half of the season										
	A320	G605	II539	S62	Average all four	A291	G626	II521	S38	Average all four
February	6.3	3.4	3.8	4.8	4.6	4.3	3.4	1.9	5.3	3.7
March	4.8	3.4	5.3	4.8	4.6	2.9	3.4	2.4	3.8	3.1
April	4.3	3.8	3.8	6.7	4.7	3.4	4.8	3.4	4.3	4.0
Average 2nd half	.....	.....	.....	.....	4.6	.....	.....	.....	.....	3.6
Average entire season	.....	.....	.....	.....	5.1	.....	.....	.....	.....	3.8

\* Through a misunderstanding this cow was sold soon after the December samples were taken—G605 was substituted for her so that the values for January to April inclusive are for 605.

NOTE.—The initial letter prefixed to each cow's number indicates the breed.

## RESULTS

The values obtained are set forth in table 1. The difference of 1.3 mgs. per liter in the zinc content of the milks from the two groups is statistically significant. A smaller difference was noted in the second half of the season than in the first, but this also is significant. It is believed that the difference between the groups in the second half would have been greater if Cow G632 had been retained in the herd. Rather consistently low values for zinc were obtained from analysis of the milk samples from the substitute Cow G605. Her milk also had a consistently low total ash content for a Guernsey (an average of 0.68% for the four months she was in the group as compared with 0.74% for her breed mate, G626, for the same four months). Also her milk looked abnormal, suggesting the high color of colostrum, although not above 4.7% fat at any time during the whole four months and running as low as 4% in March and April.

It is definitely suspected, therefore, that here was an abnormal milk, and if in consequence it be eliminated, the difference in zinc content of the milks in the second half of the trial is raised to about the same magnitude as it was in the first half, *i.e.*, approximately  $1\frac{1}{2}$  mgms. per liter more zinc in the milk when the cows were fed zinc oxide than when they were not.

In agreement with the work of Birekner (2) considerable individual variation is noted in the amounts of zinc from the different cows, but it should be observed that out of twenty-four comparisons, zinc was higher in the milk from the control group in only two instances and in one of these two the apparently abnormal milk already referred to was involved.

The amounts of zinc in the control samples are in good agreement with those reported by Birekner (2) and by Sato and Murata (9).

## SUMMARY

Zinc oxide was fed as a supplement (10 grams per cow daily) to the ration of eight cows for a period of three months by the double reversal method. The milks from the cows were analyzed for their zinc content and it was found that feeding the zinc supplement had consistently raised the level of that element in the milk, the average being 5.1 mgms. of zinc per liter of milk as contrasted with an average of 3.9 mgms. when the cows were on a control ration.

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# THE DIGESTIBILITY OF KOREAN LESPEDEZA HAY AND GROUND KOREAN LESPEDEZA SEED FOR DAIRY HEIFERS\*

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Korean lespedeza is playing an increasingly large part in the feeding program of farm animals. It is widely used as a fine mid-summer pasture crop and as hay; and the abundant yield of seed can be utilized as a high protein concentrate for livestock feeding. Although the high feeding value of Korean lespedeza has been shown often in comparative feeding trials, few reports have appeared concerning the digestibility of the hay fed alone. The investigation reported herein was therefore instituted for the purpose of determining the digestibility of different types of Korean lespedeza hays and the digestibility of Korean lespedeza seed fed with different hays.

## MATERIALS AND METHODS

Three lots of Korean lespedeza hay were used. Each was grown on a different farm near Columbia, Missouri. The fertility of the soil on which each was grown was only fair. The fields had been clipped while the lespedeza was small so that the hays were practically pure lespedeza. One lot, secured in 1941, was cut about two weeks previous to the start of blooming. In 1942 one lot was made just as blossoms were starting to appear and a second lot was made when blooming was over and the seeds were mostly in the dough stage. The alfalfa hay was third-cutting Missouri-grown hay of good quality.

The Korean lespedeza seed was cleaned seed secured on the open market.

Holstein-Friesian heifers 18 to 20 months old were used as experimental animals. The digestibility of each ration was determined with four heifers. Collection periods were of 10 days duration following a 10-day preliminary feeding period in which a constant daily amount (16 pounds) of the ration was fed.

The hays were chopped in a hammermill using a 1-inch screen. The seed was twice ground through a  $\frac{3}{8}$ -inch screen of the hammermill. The entire amount of hay or hay and seed to be fed was thoroughly mixed and sacked into daily rations previous to starting the trials. At the time of sacking the feeds were sampled for moisture determination. The composition of the feeds was determined from composite samples representing the entire lot. The lespedeza hay and seed rations were composed of 1 part seed and 3 parts hay. Four parts of alfalfa hay were used per part of seed.

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TABLE 1  
Average composition of feeds used in digestion trials and of feces, dry basis

	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract	Lignin	Cellulose	Other carbohydrates
	%	%	%	%	%	%	%	%
(1) 1941 lespedeza hay (early) .....	12.42	3.16	5.33	32.17	46.92	15.93	30.96	32.20
(2) 1942 lespedeza hay (intermed.) .....	11.84	2.37	5.48	34.09	46.32	19.97	34.50	25.84
(3) 1942 lespedeza hay (late) .....	13.28	2.76	5.45	37.02	41.49	23.14	33.13	22.24
(4) 1942 alfalfa hay .....	16.94	1.95	10.07	32.82	38.21	11.42	33.86	25.75
(5) 1941 lespedeza seed .....	36.39	0.95	5.80	10.28	37.58	5.67	13.29	28.89
(6) 1942 lespedeza seed .....	39.42	5.67	6.21	13.88	34.83	10.33	15.39	22.98
(7) Lespedeza seed + early hay .....	18.58	4.91	5.44	26.55	44.52	13.29	26.43	31.36
(8) Lespedeza seed + late hay .....	20.02	3.51	5.65	31.06	39.76	19.82	28.50	22.49
(9) Lespedeza seed + alfalfa .....	21.61	2.73	9.27	28.88	37.51	11.20	30.02	25.17
Feces from (1) .....	14.40	5.33	10.50	35.08	34.68	31.08	27.13	11.55
Feces from (2) .....	13.28	4.30	9.90	32.67	39.85	34.88	27.46	10.18
Feces from (3) .....	13.86	4.39	9.38	32.78	39.59	36.58	25.58	10.21
Feces from (4) .....	14.14	4.49	14.32	37.30	29.76	25.66	29.06	12.33
Feces from (5) .....	15.63	6.62	15.83	30.33	31.59	28.66	25.15	8.11
Feces from (6) .....	15.44	5.33	9.67	32.74	36.82	34.01	26.03	9.52
Feces from (7) .....	15.00	5.42	13.14	34.21	32.23	27.51	28.81	10.12

The feces were collected by an automatic separator patterned after an apparatus described by Ritzman and Benedict (7). A 3 per cent aliquot sample was taken from each day's fecal output from each heifer. The daily samples were composited, moistened with 2 per cent hydrochloric acid, and stored in a refrigerator at 5° C. until the completion of the collection period. The feces were then thoroughly mixed, dried with stirring in a Freas electric drying oven at 50° C., allowed to reach weight equilibrium in the laboratory, ground in a Wiley mill, and sampled for analysis.

Analyses were made of the feeds and feces by methods prescribed by the A.O.A.C. (2) for nitrogen, ether extract, crude fiber, ash, and nitrogen-free extract. Moisture was determined by drying in a drying oven at 105° C. until weighings at 2-hour intervals showed no further loss. Lignin, cellulose, and other carbohydrates were determined according to the method of Crampton and Maynard (3) except that the ether-extracted sample was moistened and autoclaved previous to digestion with 0.5 per cent pepsin in N/10 hydrochloric acid.

Digestion coefficients, total digestible nutrients, and nutritive ratios have been determined in the usual manner.

#### RESULTS AND DISCUSSION

The composition of the feeds and rations fed and the average composition of the feces from each ration are listed in table 1 on the dry basis. These show that lespedeza hay does not decrease in protein content with advancing maturity but that lignification is quite marked as the plant matures. The high protein content of lespedeza seed is demonstrated. The seed also has a sizeable portion of fiber due to the hulls which normally adhere tightly to the seed.

The average digestion coefficients, total digestible nutrients, and nutritive ratios which were calculated for the different rations and feeds are presented in table 2.

The effect of advanced maturity upon the nutritive value of Korean lespedeza hay is clearly shown in table 2. With increased maturity there was a decrease in digestibility of practically every nutrient division. While lespedeza hay cut well in advance of bloom was very acceptable as a source of total digestible nutrients, late cut hay was markedly inferior. It is also worthy of note that the nutritive ratio of the good lespedeza used here was much wider than the 1:4.7 estimated by Morrison (6). This is due to the fact that the protein of Korean lespedeza hay is not digested as thoroughly as that from many other legumes. The high lignin content of lespedeza leaves has been proposed by Swanson and Herman (9) as an explanation of the poorer protein digestion from lespedeza hay.

The data in table 2 also present further evidence of the importance of the lignin content of the feed to its utilization. There is a remarkably close

TABLE 2  
*Digestion coefficients, total digestible nutrients, and nutritive ratios of Korean lespedeza hay and seed*

	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Lignin	Cellulose	Other carbohydrates	Total dry matter	Total dig. nutr. (dry basis)	Nutritive ratio 1:
	%	%	%	%	%	%	%	%	%	
941 lespedeza hay (early) .....	49.17	29.15	54.21	68.90	17.99	63.18	84.91	57.95	57.95	8.48
942 lespedeza hay (intermed.) .....	42.51	7.06	50.82	55.78	10.40	59.16	79.79	48.70	48.51	8.64
942 lespedeza hay (late) .....	41.03	10.18	49.92	46.03	10.60	56.34	74.01	43.44	43.86	7.01
942 alfalfa hay .....	65.10	5.00	52.42	67.47	5.99	64.09	79.96	58.17	54.23	3.92
Lepedeza seed + early hay .....	63.51	41.49	50.41	69.26	6.62	58.69	88.76	56.64	60.59	4.13
Lepedeza seed + late hay .....	61.70	24.59	47.57	53.99	14.80	54.58	78.87	50.29	50.54	3.09
Lepedeza seed + alfalfa hay .....	72.52	21.34	53.11	65.96	3.00	61.99	84.06	60.39	57.06	2.64
Lepedeza seed (- early hay) .....	77.66	53.85	20.82	70.56	0	27.39	94.94	52.86	68.76	1.43
Lepedeza seed (- late hay) .....	81.74	44.86	30.28	81.29	41.90	43.52	90.13	70.01	70.45	1.19
Lepedeza seed (- alfalfa) .....	84.66	42.66	59.33	59.67	1.85	44.45	97.84	68.87	67.83	1.03
Average of all lespedeza seed .....	81.35	46.79	36.81	70.51	14.58	38.45	94.30	63.91	69.01	1.22

relationship between the lignin content and the digestibility of the three lots of lespedeza hay. The lignin-digestibility relationship does not seem the same for the alfalfa hay as for the lespedeza hay, however. This may be due to the fact that the heifers did not ruminate on the alfalfa rations, presumably because it was ground too fine, but did ruminate on all of the lespedeza hay rations. This lack of rumination may have slightly depressed the digestibility of the alfalfa hay. Ritzman and Benedict (8) found that ground alfalfa hay was less digestible than unground hay. It is also probable that the type and manner of deposition of lignin is so different in alfalfa and lespedeza that their relative amounts are not an adequate measure of their effect upon digestibility of the two kinds of hay.

A few seeds from the late-cut lespedeza hay came through the heifers whole. This loss of nutrients may have accounted for part of the low digestibility of the late-cut hay. The undigested seeds were not in great numbers, however, and it is believed that their effect upon lowering the digestibility of the hay was of minor importance compared to the effect of the high degree of lignification of the plant.

In drawing conclusions about the effect of maturity upon the digestibility of Korean lespedeza hay from these data, one must consider that the three lots of hay came from three different farms and were grown in two different seasons. Hays of different maturity from the same field grown in the same season may show slightly different variations than are shown in table 2.

The digestibility of Korean lespedeza seed was determined with three lots of hay, early-cut lespedeza hay, late-cut lespedeza hay, and alfalfa hay. The average results of the trials with each hay and the average of all of the lespedeza seed digestibility calculations are presented in table 2. It is shown there that the crude protein and the non-fibrous carbohydrate of lespedeza seed are quite highly digestible. Great variation was found in the digestibility of the fibrous portion of the seed. The seed was moderately high in total digestible nutrients, 69.01 per cent on a dry basis, and furnished a large amount of digestible protein, the average nutritive ratio being 1:1.22. The differences in digestibility of the seed fed with the different hays were not of noteworthy importance when it is considered that two different lots of seed were used. The 1941 seed was higher in ether extract and its ether extract was digested better than that of the 1942 seed.

Many analyses of lespedeza seed at the Missouri station have shown that it contains approximately 35 to 40 per cent total crude protein. This would be equivalent to about 28.5 to 32.5 per cent digestible crude protein. The ground lespedeza seed was eaten with relish by every one of the eight heifers used. It did not appear to exert any abnormal effect upon the animals. Experiments by Herman and Ragsdale (4), Irwin and Kempster (5), and the Missouri Department of Animal Husbandry (1) have shown that Korean lespedeza seed serves satisfactorily in rations of dairy cattle, beef cattle,

poultry, and sheep. It should therefore be considered as a very nutritious high protein concentrate which well deserves the attention of farmers seeking to increase their supply of home-grown high protein feeds.

#### SUMMARY

Digestion coefficients were determined on Korean lespedeza hay cut well in advance of bloom, just before bloom, and after bloom. The digestibility of practically all nutrients was decreased with advanced maturity and the late-cut hay was a very poor source of total digestible nutrients. The lignin content of the hays increased with maturity and as the lignin increased digestibility was decreased.

Digestion coefficients were calculated for ground Korean lespedeza seed fed with two types of lespedeza hay and with alfalfa hay. The lespedeza seed crude protein averaged 81.35 per cent digestible which makes the seed contain approximately 28.5 to 32.5 per cent digestible crude protein. The seed contained an average of 69.01 per cent total digestible nutrients and had a nutritive ratio of 1:1.22.

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## CITRUS MOLASSES—A NEW FEED\*

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Citrus molasses was produced in Florida on a commercial scale during the canning season of 1941–1942 with a production of 2500 tons. During 1942–1943 this was increased to about 4700 tons. Approximately one-half of domestic blackstrap molasses was unavailable for feeding purposes due to its retention for manufacture of war materials. Hence citrus molasses filled a need in the manufacture of mixed feeds. The supply of citrus molasses has been insufficient to meet the demand of the mixed feed industry.

### RECOVERY OF CITRUS MOLASSES

From a survey made by the Department of Agricultural Economics of the Florida station it was determined that 67.5 per cent of bulk citrus fruits remains as peel, rag, and seed, from the canning of hearts, juice, and citrus concentrate. This residue or fresh pulp contains about 85 per cent of moisture mostly as bound water, or water of constitution.

Addition of calcium salts to the fresh pulp liberates the bound water. One-half of the water then is removed by pressure. The pulp is dehydrated into dried citrus pulp. The press juice contains about 5.4 per cent solids—mainly sugars. Evaporation of this material under partial vacuum to about one-thirteenth of the original volume results in a light colored sweet viscous syrup known to the feed industry as “citrus molasses.” A glucoside—naringin—which imparts a characteristic flavor to citrus peel, also is concentrated in the process, so that its flavor is accentuated in the molasses.

### COMPOSITION OF CITRUS MOLASSES

Citrus molasses is not wholly standardized as to composition perhaps due to changes in the press juice from citrus fruits as the season advances. The producers concentrate it to a reading of 75 degrees Brix. The resulting product varies slightly in total solids content, and in proportions of the different constituents.

Four analyses of citrus molasses are available, 3 from the 1941–1942 crop, and one (Sample No. 4) determined in the Nutrition Laboratory from the 1942–1943 crop. These analyses are given in table 1.

The specific gravity of citrus molasses (Sample No. 4 was 1.376) and a pH value of 4.65 was observed. Analyses showed 0.94 per cent of calcium citrate [ $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)$ ] present as fine white flakes which settled out on

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TABLE 1  
*Analyses of citrus molasses*

Sample No.	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Reducing sugars	Non-reducing sugars	Weight per gallon
	%	%	%	%	%	%	%	%	lbs.
1	66.8	5.00	0.15	None	57.97	3.57	20.92	19.87	11.07
2	71.2	3.86	0.24	0.03	61.76	5.31	27.74	15.28	11.61
3	75.2	3.75	0.23	None	65.95	5.24	32.00	18.70	.....
4	66.5	3.39	None	None	59.64	3.47	.....	.....	11.17

standing. The major constituents of this molasses are reducing and non-reducing sugars, the proportions of which vary. No fiber is present. The amount of ether extract depends upon the mechanical separation, or the evaporation of citrus oils.

No digestion trials have been conducted using citrus molasses. Digestion trials on similar products included 4 with beet molasses and 26 with cane or blackstrap molasses, cited by F. B. Morrison in "Feeds and Feeding," 20th edition. The weighted average of these digestion coefficients—35 per cent for crude protein and 90.1 per cent for nitrogen-free extract—applied to the average crude protein and nitrogen-free extract from analyses of citrus molasses in table 1, gave an estimated value of 1.4 per cent digestible crude protein and 56.7 per cent of total digestible nutrients, with 69.9 per cent of dry matter in the molasses.

#### PALATABILITY OF CITRUS MOLASSES

Since the flavor of naringin predominates in citrus molasses, the palatability of the product for dairy cows was questioned. Two brief conclusive palatability trials were conducted with the Jersey cows in the Experiment Station dairy herd using different levels of molasses. Since a 9 per cent level of blackstrap (sugarcane) molasses is the amount present in many commercially mixed dairy feeds five per cent of citrus molasses was incorporated into home-mixed concentrates. Twenty-five cows were offered 2 pounds each of the molasses-concentrates after consuming their regular afternoon allowance of feed. All of the feed was consumed without hesitation. On the second day, 10 pounds of citrus molasses were incorporated with 90 pounds of concentrates; offered in the same manner, and consumed completely by the 25 animals.

Thirty-four Jersey and Guernsey cows were offered straight citrus molasses from one to three times while in the stanchions. Twenty-six animals refused it on first offering, 17 on the second, and 8 on the third offering. Eleven cows ate all of the citrus molasses; 7 took part; 5 tasted it, and three others ate all when mixed feed was sprinkled beside it. When offered first, the majority of cows did not care for the pure product, the proportion decreasing on later offerings. It is believed that cows would learn to take this feed, but it appears undesirable for feeding separately.

#### EFFECT ON MILK FLAVOR

Does citrus molasses affect the flavor of milk? To answer this question individual milk samples were obtained from four Jersey cows after being in dry lot 10 hours without feed. On other days, citrus molasses was added as 10 per cent of the mixed concentrates, fed 2 hours prior to milking time, and additional milk samples obtained. Samples from the complete milking were collected in milk bottles, cooled immediately in ice water and held at 35° F.

TABLE 2

*Effect of citrus molasses on the flavor of milk, as scored by 3 judges\**

	Cow No.				Average
	664	655	708	737	
<i>Cows received no feed 10 hours prior to milking; 2 trials</i>					
Average score .....	38.1	37.7	38.7	38.7	38.3
<i>Cows received molasses-concentrate feed 2 hours prior to milking; 4 trials</i>					
Average score .....	35.1	38.1	37.2	38.0	37.1

\* The samples were scored according to the American Dairy Science Association score card, which allows 45 points for flavor.

for 15 hours. The samples then were pasteurized at 143° F. for 30 minutes; cooled to 60° F., and scored for flavor by 3 experienced judges.

The average flavor score of milk obtained when no feed was consumed by the cows 10 hours before milking, was only 1.2 points higher than when mixed concentrates containing 10 per cent of citrus molasses were fed 2 hours before milking. At least part of this difference in score can be attributed to the concentrate mixture itself. Also, the milk from cow No. 664 increased in saltiness during the course of the experiment. While some feed flavors were noted, they were neither intense nor objectionable. It is believed that if this product was fed at milking time, no noticeable effect on flavor of milk would result.

## CITRUS MOLASSES IN GRASS SILAGE

Blackstrap molasses is used in ensiling grasses and legumes. In its stead, a trial was conducted using Napier grass (*Pennisetum purpureum* Schumach), ensiled without molasses, 2 levels of citrus molasses with Napier grass and one level with pigeon peas (*Cajanus indicus* Spreng.). The levels of citrus molasses used with the forage are shown in table 3.

The relative palatability of these four silages was observed with nine Jersey heifers, based upon the number of animals eating each silage, and the heifers showed a decided preference for the molasses-Napier over the

TABLE 3

*Effect of ensiling Napier grass and pigeon peas with citrus molasses, upon its palatability to Jersey heifers*

Forage	Proportion of citrus molasses added	Aroma of silage	pH	Preference of heifers
	%			
Napier grass .....	0	Fair to good	4.08	3rd
“ “ .....	2	Excellent	3.86	1st
“ “ .....	4	Excellent	3.95	2nd
Pigeon peas .....	4	Good	5.08	4th

plain Napier silage. Although the pigeon pea silage rated fourth in palatability, it ensiled well with the added citrus molasses, and had a desirable aroma.

#### SUMMARY AND CONCLUSIONS

Citrus molasses is replacing cane molasses in part of the mixed dairy feeds. Over 4700 tons were obtained in the second year of operation, it being recovered along with dried citrus pulp as by-products of the citrus canning industry. Reducing and non-reducing sugars constitute two-thirds of the dry matter. No fiber, little fat, 4 per cent of crude protein, and over 0.9 per cent of calcium citrate were present. Values of 1.4 per cent of digestible crude protein and 56.7 per cent of total digestible nutrients were estimated by applying the digestion coefficients for beet and cane molasses.

Dairy cows found citrus molasses highly palatable mixed in concentrates at 5 and 10 per cent levels, but much less so when first offered separately. A slight feed flavor was imparted to milk when fed 2 hours before milking time. The flavor was neither intense nor objectionable.

Additions of citrus molasses to a non-saccharin grass (Napier) at 2 and 4 per cent levels improved aroma and palatability of the silage. Pigeon peas ensiled satisfactorily with a 4 per cent addition of citrus molasses.

#### ACKNOWLEDGMENTS

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# THE EFFECT OF FEEDING COTTONSEED MEAL AS THE ONLY CONCENTRATE ON SEVERAL PROPERTIES OF MILK.

## I. FAT, TOTAL SOLIDS AND ASH CONTENT\*

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Cottonseed meal has been one of the most commonly used protein supplements for dairy cows. In the southern states where this meal is the principal locally-produced concentrate feed, it is usually the cheapest source not only of protein but also of total digestible nutrients available to the dairyman. Therefore, it is often economical to feed cottonseed meal as the only concentrate to dairy cows.

Research work at various stations (5, 7, 9, 10, 13, 14, 16) indicate that cottonseed meal is a satisfactory concentrate for dairy cows when it is supplemented with adequate amounts of vitamin A and calcium.

It is recognized that many factors may cause variations in the composition of milk. The results of numerous experiments indicate that the fat, the most variable constituent of milk, cannot be altered permanently to any marked degree when the cow receives what is considered as an adequate ration, but many feeds of high oil content, including cottonseed meal, have been shown to cause at least a temporary increase in the fat percentage (1, 2). In contrast prickly pear and certain oils, such as cod liver oil, have been found to decrease the fat percentage (11, 21). Furthermore, it is extremely difficult to relate directly the variations in the solids-not-fat and the ash of the milk to the feed of the cow. Taylor and Husband (20) and other workers have shown that feed can alter the composition of milk indirectly through an effect on the milk production. The percentage of total solids, of fat and of ash usually is found to vary inversely and the percentage of lactose directly with the volume of milk yielded. Recently Riddet *et al.* (18) have confirmed observations that subnormal feeding results in a decrease of the percentage of solids-not-fat in milk along with a reduction in volume produced.

Since general economic and nutritional conditions in the South indicate the need of conserving and utilizing all the constituents of milk for human consumption, this study was concerned primarily with the effect of cottonseed meal on the milk solids collectively as well as individually.

### EXPERIMENTAL

The effects of feeding cottonseed meal as the only concentrate to dairy cows were studied by comparing the properties of the milk produced by a

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group of cows receiving cottonseed meal (plus 2 per cent bone meal and 1 per cent salt) with the properties of milk produced by a similar group receiving a concentrate mixture of 400 pounds corn gluten meal, 200 pounds wheat bran, 200 pounds ground corn, 200 pounds ground oats (plus 2 per cent bone meal and 1 per cent salt). The roughage received was the same for the two groups, either corn and soybean silage or pasture.

The cows used in this experiment were carefully selected from the Holstein herd so that each cow on the cottonseed meal ration was paired with a comparable cow on the control ration. An attempt was made to maintain from eight to ten cows in each group; however, at times the number dropped to five cows per group.

A comparison of the milk from the two groups as to total solids, fat and ash content (4) over a period of sixteen months is presented in figure 1.

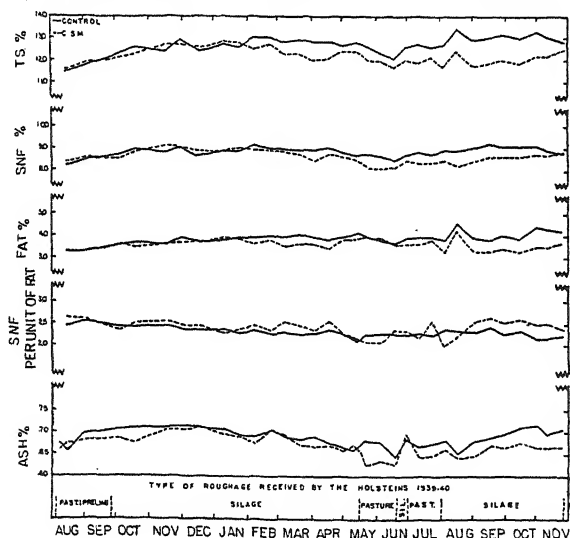


FIG. 1. The total solids, solids-not-fat, fat, ratio of fat to solids-not-fat and ash in the milk produced by a group of cows receiving cottonseed meal as the only concentrate compared with the same constituents in the milk produced by a similar group receiving a normal concentrate mixture.

During the first four months the groups were receiving their respective rations, no differences were evident in the fat and total solids content of the milks. After this time, the milk produced by the cows receiving the cottonseed meal began to have a consistently lower fat and total solids content than the milk from the control group. An exception to this difference in fat content occurred for a brief time when the cows were released on pasture in May.

The solids-not-fat content of the milk from the two groups follows the same trend as the total solids and fat. The ratio of the solids-not-fat to fat



indicates that there was a tendency for the fat to be lower in proportion to the solids-not-fat in the milk from the cottonseed meal group than in that from the control group. This tendency did not hold true during the period the roughage was being interchanged with silage and pasture.

The ash content of the milk from the two groups of cows showed no significant differences until the cows were turned on pasture. This change in roughage was accompanied by a drop in the ash of the milk from the cows receiving the cottonseed meal. The ash increased when the cows were returned to silage, but dropped again when returned to pasture and then remained low even though the cows were once more fed silage.

#### DISCUSSION

It may be significant that four months on the rations elapsed before any differences in total solids and fat became evident. The differences in the composition of the milk from the two groups showed no apparent correlation with the quantity of milk produced (unpublished data) except for the higher fat and lower production from the cottonseed meal group at the time the cows were first turned on pasture in May. At this stage, several individuals of the cottonseed meal group underwent a temporary physiological upset characterized by anorexia, decreased milk production and abortions. At the same time, the production of the control group increased and the general health of the animals remained good. Under these conditions variations in the composition of the milk were to be expected. The question as to whether the effects on the cows in the test were related to the cottonseed meal ration or to some other factor or factors coincidental with the progress of the experiment remains unanswered. There was no definite indication that the cows were suffering at any time from either a vitamin A or a calcium deficiency.

After the differences in composition of the milk from the two groups had become established, it was planned to reverse the groups, but so many cows had to be removed from the cottonseed meal ration that the group became too small to continue. Another paired series, using Jerseys, was placed on a similar regime. After a period of about four months, the same differences in fat and total solids observed in the milk of the Holstein groups became evident in the Jerseys; but since still more difficulty was experienced in maintaining the organization of these groups, the results cannot be accepted as definite confirmation of the data reported for the Holsteins.

Hills *et al.* (12) and Perkins (17) concluded from their investigations that the level of protein feeding does not affect the composition of milk. On the contrary, Stewart and Tocher (19) observed a decrease in percentage of solids-not-fat from feeding a high protein ration, and Keith *et al.* (15) reported a lower fat and specific gravity in the milk produced on a ration of cottonseed meal and prairie hay than in the milk produced on a standard

herd ration. Although there were other factors involved in the experiment reported in this article, the results indicate that the level of protein feeding might affect the composition of the milk.

The differences in ash content of the milk from the two groups tended to follow the same trends as the differences in solids-not-fat. Becker *et al.* (6) found that calcium and phosphorus of milk remained normal even though the cows were suffering from a severe deficiency of these elements. This is in accord with the general belief that feed does not directly affect the ash content of milk (8). On the other hand, it has been shown that certain elements of the ash can be increased by feed (3). Interesting results might have been obtained from a study of the elemental composition of the ash of the milk from the two groups.

#### SUMMARY

The percentage of fat, total solids and ash of the milk produced by a group of Holstein cows receiving cottonseed meal as the only concentrate was compared with that of a similar group receiving a mixture of corn gluten meal, wheat bran, ground corn and oats. The roughage was the same for the two groups. The data collected covered a feeding period of sixteen consecutive months.

Four months after placing the animals on their respective experimental rations the milk produced by the group receiving the cottonseed meal ration had a lower percentage of total solids, fat and solids-not-fat than that from the control group. Later the ash content likewise became lower.

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# THE EFFECT OF FEEDING COTTONSEED MEAL AS THE ONLY CONCENTRATE ON SEVERAL PROPERTIES OF MILK.

## II. NITROGEN DISTRIBUTION\*

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Investigations (2, 5, 9, 11) have shown that feeding a high level of protein increases the non-protein nitrogen content of the milk. Although this is generally accepted to be the only direct effect of feed on the nitrogen composition of milk, some investigators (3, 10) have observed a slight variation in other nitrogen fractions accompanying changes in the level of protein feeding.

In a preceding report (4) the total solids, the fat and the ash content of the milk produced by a group of cows receiving cottonseed meal as the only concentrate were compared with the content of the same constituents in the milk produced by a comparable group of cows receiving a normal concentrate mixture. Since any major change in the nitrogen fractions of milk may be expected to influence its manufacturing characteristics, an additional study of the properties of the milk from the foregoing groups included determinations of the nitrogen distribution of the milk.

### EXPERIMENTAL

The total nitrogen, the casein nitrogen, the non-casein protein nitrogen and the non-protein nitrogen (7) in the milk produced by the two groups of cows are presented in figure 1.

Immediately after placing the cows on their respective concentrate rations, the non-protein nitrogen in the milk from the cottonseed meal group increased; whereas that from the control group decreased slightly. For the most part, this difference was uniform throughout the remainder of the experimental period. This parallelism was maintained even though there were variations from time to time in the level of the non-protein nitrogen. When the animals were released on pasture, this nitrogen fraction increased, and when silage feeding was resumed, it decreased.

The non-casein protein nitrogen fraction of the milk from the two groups showed no difference that could be attributed to the rations.

During the first four months, no differences were evident in the casein nitrogen content of the two milks. After this period, when a difference in fat and total solids was observed (4), the milk from the cottonseed meal group had a lower casein nitrogen content than the milk from the control

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group. This difference was accentuated when the cows were turned on the pasture, particularly during the first grazing period. The precipitous drop in this constituent noted during this period was associated with a change in the physiological condition of several cows in the cottonseed meal group (4).

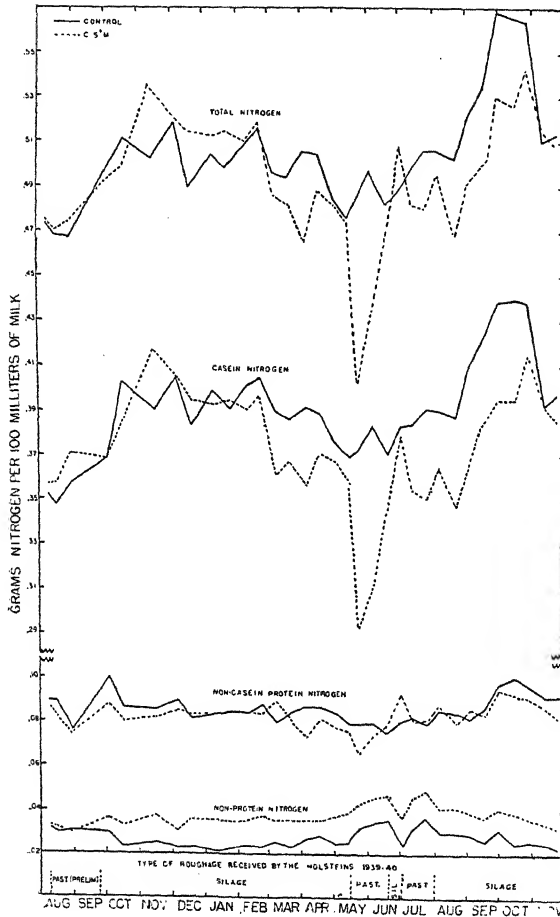


FIG. 1. The amount of total nitrogen, casein nitrogen, non-casein protein nitrogen and non-protein nitrogen in milk produced by a group of cows receiving cottonseed meal as the only concentrate compared with the same nitrogen fractions in the milk produced by a comparable group of cows receiving a normal concentrate mixture.

Apparently the differences in the level of the total nitrogen in the milks from the two groups were due primarily to the relative levels of the non-protein nitrogen and the casein nitrogen fractions. The increase in non-protein nitrogen in the milk from the cottonseed meal group tended to increase the total nitrogen to a higher level than that in the milk from the

control group until changes in the casein nitrogen began to counterbalance the effects of the non-protein nitrogen. The reduction of the casein nitrogen in the milk from the cottonseed meal group apparently was sufficient to offset the higher non-protein nitrogen. Hence, the total nitrogen was first

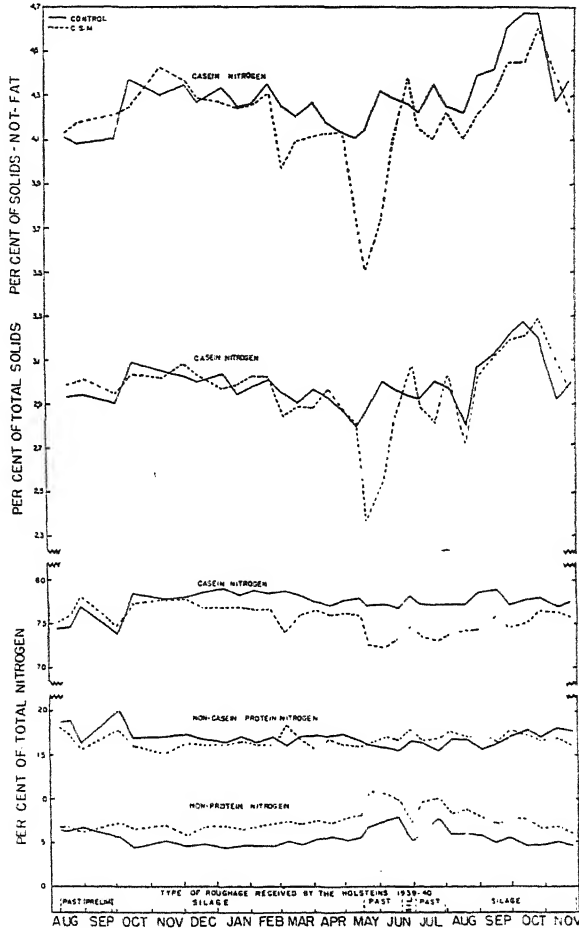


FIG. 2. The per cent of total nitrogen as casein, non-casein protein and non-protein nitrogen, and the per cent of total solids and of solids-not-fat as casein nitrogen in the milk produced by a group of cows receiving cottonseed meal as the only concentrate compared with the same nitrogen distribution in the milk produced by a comparable group of cows receiving a normal concentrate mixture.

higher and then lower in the milk from the cottonseed meal group than in the milk from the control group.

The two sets of curves for casein nitrogen, figure 2, show that this fraction calculated as per cent of the solids-not-fat follows the same trend

and shows the same general group relationships as it does when expressed in terms of grams per 100 milliliters of milk, figure 1. However, when expressed on a basis of per cent of the total solids in the milk, the aforementioned apparent differences between the two milks was not in evidence, except for the period when the cows were physically upset. This indicates that the fat (4) in the milk decreased sufficiently to keep the ratio of casein nitrogen to total solids in the milk from the cottonseed meal group about equal to the same ratio for the milk from the control group.

The remaining three sets of curves presented in figure 2 show the distribution of the total nitrogen as casein, non-casein protein and non-protein nitrogen. The lower percentage of casein nitrogen from the cottonseed meal group, as compared with that of the control group, is due partly to a diluting effect of the increased non-protein nitrogen and partly to a definite lower casein in relation to the solids-not-fat.

#### DISCUSSION

The high non-protein nitrogen observed in the milk from the group receiving cottonseed meal is in agreement with reports (2, 5, 9, 11) that the level of protein feeding affects directly the amount of non-protein nitrogen present in the milk.

Most of the published reports indicate that the protein nitrogen in the milk is not affected by the level of protein feeding, although a slight increase has been observed. Stewart and Tocher (10) reported that albumin and globulin were increased but that casein was not affected. Hills *et al.* (3) found a slight increase in the casein-albumin fraction with an increasing level of protein feeding. The results reported in this article indicate that the albumin and the globulin apparently were not affected by feed; whereas casein instead of being increased actually was lowered by cottonseed meal feeding.

Riddet *et al.* (6) observed that casein was decreased by subnormal feeding, but the cows in the experiment reported in the present article hardly could be considered on a subnormal ration in terms of total digestible nutrients. Davies (1) found that milk low in solids-not-fat was also low in casein and especially low in lactose but high in non-protein nitrogen, albumin, and globulin nitrogen. If the amount of lactose is calculated from the results reported here (4), the doubtful condition of both a low lactose content and a low ash is shown in the milk from the cows receiving cottonseed meal as compared with milk from the control group.

Using Rowland's (8) value of 77.0 as the lower limit for casein nitrogen, expressed as per cent of total nitrogen for normal milk, the cows on the cottonseed meal ration could be considered as secreting abnormal milk after being on this ration about three months. Rowland has indicated that milk of this type is characteristic of that from cows suffering from sub-



clinical mastitis. Although no special study was made regarding this point, there were no apparent indications that the cottonseed meal group of cows was any more susceptible to udder troubles than the control group.

Results from the groups of Jerseys mentioned in a previous article (4) tended to confirm the effects of cottonseed meal feeding on the non-protein and casein nitrogen of milk as reported in this article.

#### SUMMARY

The nitrogen distribution of the milk produced by a group of cows receiving cottonseed meal as the only concentrate was compared with that of milk from a comparable group receiving a concentrate mixture of corn gluten meal, wheat bran, and ground corn and oats. The roughage was the same for the two groups.

The milk from the cottonseed meal group had a higher non-protein nitrogen content than that from the control group.

The two systems of feeding resulted in no apparent differences in the non-casein protein nitrogen content of the milk.

About four months after placing the cows on their respective experimental rations, the group receiving cottonseed meal as the only concentrate began to produce milk of a lower casein nitrogen content than did the control group.

The comparatively lower casein nitrogen was accompanied by a lower fat and total solids content in such a ratio that the casein nitrogen expressed as per cent of total solids was the same for both groups.

Differences in the total nitrogen content of the two milks were dependent on the relative levels of non-protein and casein nitrogen; hence the total nitrogen was first higher and then lower in the milk from the cottonseed meal group than in the control group.

The quantity of milk produced by the two groups of cows was essentially the same; therefore the differences in properties of the milk reported in this series of articles cannot be related to the milk yields.

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THE EFFECT OF FEEDING COTTONSEED MEAL AS THE ONLY  
CONCENTRATE ON SEVERAL PROPERTIES OF MILK. III.  
pH, RENNET COAGULATION, HEAT COAGULATION  
AND CURD TENSION\*

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Any factor altering the composition of milk may be expected to affect the physical-chemical properties of the milk, while the physical-chemical properties of milk can be altered without any detectable change in milk composition. For instance, an effect on the rennet coagulation of milk may or may not be accompanied by a detectable change in milk composition (5, 6, 11, 13).

The belief that feed does not directly affect the composition of the milk has led to the general opinion that such properties as pH, rennet coagulation, heat coagulation, and curd tension are likewise not affected by the ration. Investigations have shown that acids in the ration do not alter the acidity of the milk (1, 10). Neither factors affecting the curd tension of milk (2) nor other coagulation characteristics of milk have been definitely related to the feed consumed by the cow.

In preceding reports the total solids, the fat and the ash content (8) and the nitrogen distribution (9) in the milk produced by a group of cows receiving cottonseed meal as the only concentrate were compared with the same properties in milk produced by a comparable group of cows receiving a normal concentrate mixture. The cottonseed meal group of cows produced milk of a lower solids content than the control group; hence, other properties of the milk might be expected to have been affected. Thus additional data from milk samples previously described (8, 9) are presented in this report.

EXPERIMENTAL

The hydrogen ion concentration of the milk samples was determined with a glass electrode pH meter. The rennet coagulation time was obtained by the procedure as outlined by Sommer and Matsen (12). The heat coagulation was studied by recording the time required for the milk to coagulate when sealed in small glass tubes and immersed and agitated in an oil bath at 136° C. (7). The effect of small changes in the salt balance of the milk on the heat coagulation was determined by recording the time required for coagulation of various milk samples to which calcium acetate and sodium diphosphate, respectively, had been added at the rate of 0.4 ml. of M/4 salt per 50 ml. of milk. The procedure recommended by the Committee on

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Methods of Determining the Curd Tension of Milk (3) was used for studying the curd tension of the milk samples. In addition to the determination of the maximum grams required for cutting the surface the weight for cutting the body of the curd also was recorded.

A comparison of the pH, the rennet coagulation, the heat coagulation and the curd tension of the respective milks produced by the two groups of cows is presented in figure 1.

The milk from the cottonseed meal group had a slightly but consistently higher pH than the milk from the control group during the first few months

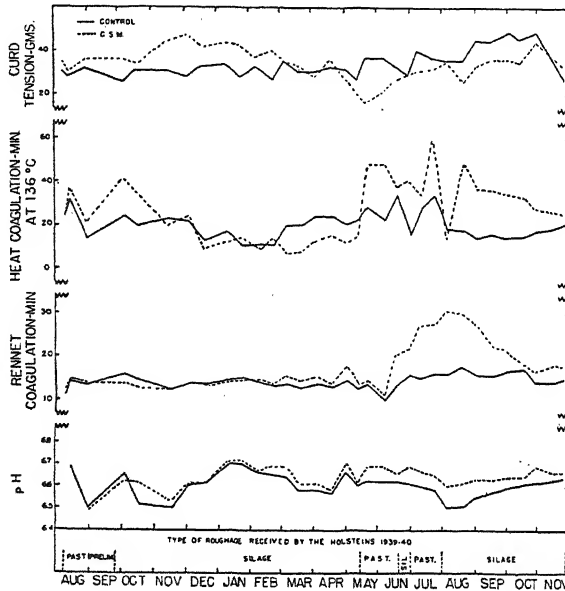


FIG. 1. The pH, rennet coagulation, heat coagulation and curd tension of the milk produced by a group of cows receiving cottonseed meal as the only concentrate as compared with the same properties of the milk produced by a comparable group receiving a normal concentrate mixture.

of the feeding trial. The change from silage feeding to pasture grazing was accompanied by a still greater spread in the pH values of the two milks, the increased difference persisting throughout the remainder of the trial.

After the first four months, when the milk from the cottonseed meal group began to have a lower solids content (8), the rennet coagulation time for this milk began to be slightly greater than for the control milk. A few weeks after turning the cows on pasture the rennet coagulation time for the milk from the cottonseed meal group increased markedly over a period of about three months and then gradually returned to near the normal level after silage feeding was resumed.

The heat coagulation of the milks showed no uniform and definite differences; however, when the cows were changed back and forth from pasture to silage, the heat coagulation of the milk from both groups fluctuated considerably, the milk from the group on the cottonseed meal ration tending to be more stable to heat than that from the control group.

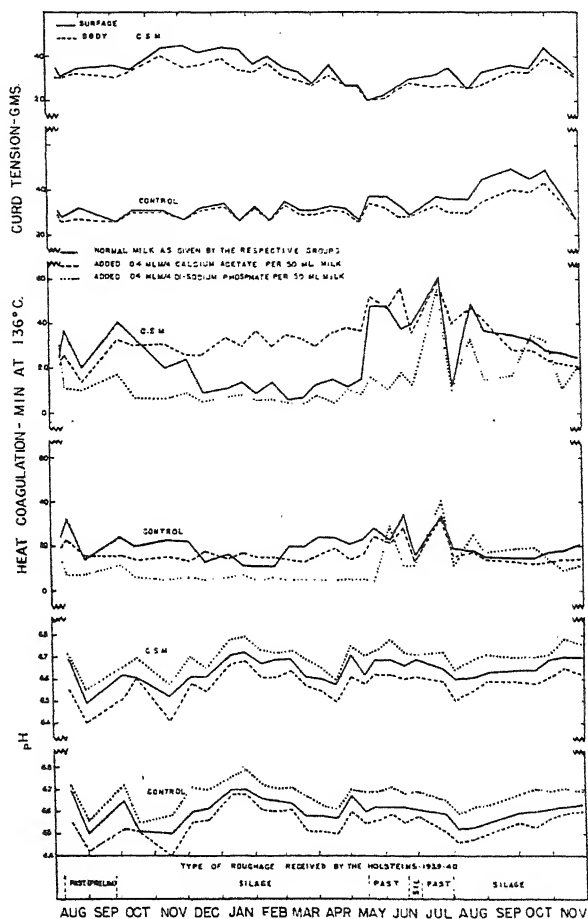


FIG. 2. The effects of added salts on the pH and the heat coagulation, and the differences between surface and body curd tension of the milks produced by the groups of cows on a cottonseed meal and control rations.

During the first few months the curd tension of the milk from the cottonseed meal group was higher, but for the remainder of the trial it was lower than that from the control group. There seemed to be a direct relationship between the lower solids content of the milk (8) and the lower curd tension. As shown in figure 2 there is a marked parallelism between the surface curd tension and the body curd tension in the respective samples of milk.

The additions of calcium acetate and of di-sodium phosphate to the milks had the same effect on the pH of the two milks, but different effects on the heat coagulation as is indicated by the curves presented in figure 2. The addition of phosphate always increased and the addition of calcium always decreased the pH of the milk samples. The heat coagulation time for the milk from the control group usually was decreased by the addition of either salt; whereas the addition of the calcium salt usually increased while phosphate decreased the heat stability of the milk from the cottonseed meal group.

#### DISCUSSION

The general differences between the milk from the cottonseed meal group and that from the control group in pH, rennet coagulation, heat coagulation and curd tension follow, for the most part, trends that could be predicted from the differences in the composition of the milks (8).

The higher pH of the milk from the cottonseed meal group in comparison with the milk from the control group is in accord with the lower solids-not-fat content of the cottonseed meal milk. Keith *et al.* (4) observed these same general differences in a comparison of milk produced by a group of Jerseys receiving cottonseed meal and prairie hay with that from a similar group receiving a standard herd ration.

The lower solids in the milk from the cottonseed meal group would account for a longer rennet coagulation time than for the milk from the control group. The difference in ash content of the two milks is also in harmony with the differences in rennet coagulation time. The marked increase in coagulation time for the group from the cottonseed meal group occurred following a physical upset of the cows on the cottonseed meal ration (8). This prolonged increase in the rennet coagulation time was probably associated with the physiological condition of the cows.

The effects on the heat coagulation of the milks from the two groups were variable, but the results revealed a difference in the heat stability. The differences in coagulation time and in composition of the comparative whole milks suggested that a difference in the heat stability of their evaporated products might be expected.

#### SUMMARY

The pH, rennet coagulation, heat coagulation and curd tension of the milk produced by a group of cows receiving cottonseed meal as the only concentrate were compared with the same properties of milk produced by a comparable group of cows receiving a normal concentrate mixture.

These properties of the milks follow the same general trend as the differences in composition of the two milks.

The milk from the cottonseed meal group had a higher pH than that from the control group. The slight difference observed during the first few months increased as the difference in total solids increased.

The milk from the cottonseed meal group required longer to coagulate by rennet than that from the control group after about the first five months of the feeding trial.

Variable differences were recorded for the heat coagulation of the milks. The data suggest a practical problem relative to the heat stability of the evaporated products produced from milk of cows receiving a high level of cottonseed meal in the ration.

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# THE EFFECT OF FEEDING COTTONSEED MEAL AS THE ONLY CONCENTRATE ON SEVERAL PROPERTIES OF MILK.

## IV. FAT CONSTANTS\*

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Numerous reports in the literature show that many feeds affect the composition of milk fat. A great number of these records are concerned with cottonseed and cottonseed products as dairy feeds. Although most of the investigations recorded are in agreement relative to the specific effects of cottonseed meal upon the fat properties, the few discrepancies noted may be explained partially on the basis of the role played by such factors as length of feeding period, type of basal ration, method of comparison, etc., on the nature and degree of alteration of milk fat.

Most of the studies of the effect of feeding cottonseed meal on milk fat composition have been for short periods, and many of the investigators have given little consideration to the type of basal ration and to the maintenance of adequate control groups of cows.

Since the composition of the fat is known to be influenced easily by feeds and since the effects of cottonseed meal feeding over a short duration of time have been fairly well established, it was deemed desirable to determine the comparative effects of protracted cottonseed meal feeding on some of the constants of the fat from the milk samples obtained under conditions previously reported (2, 3, 4). Furthermore, since the composition of the fat has been shown to be related to its susceptibility to oxidation (5), observations were made on the comparative effects of the feeds on the oxidation of filtered milk fat.

### EXPERIMENTAL

The fat samples were obtained from milk produced by two comparative groups of cows, one of which received cottonseed meal as the only concentrate, while the other received a normal grain mixture. The respective milk samples were pasteurized and churned. The resulting butter was then melted and filtered for subsequent study.

The saponification number, iodine number, Reichert-Meissl number, butyro-refractometer degrees, stability value (5) and acid degree of the fat samples were determined. The data obtained are presented in figure 1 and in table 1.

The milk fat from the cottonseed meal group had a lower saponification number than that from the control group, a relationship which would be

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predicted from a review of the literature. However, for a short period during the sixth and seventh months of the feeding trial this difference was practically nil. It is not known whether the latter deviation from the expected results was due to the ration or to some other cause.

For most of the feeding period the milk fat from the cottonseed meal group had a higher iodine number than that of the control group. During the seventh month, the iodine number, as did the saponification number, failed to show the expected difference in the two milk fats. While the cows were being transferred back and forth from silage to pasture, the differences

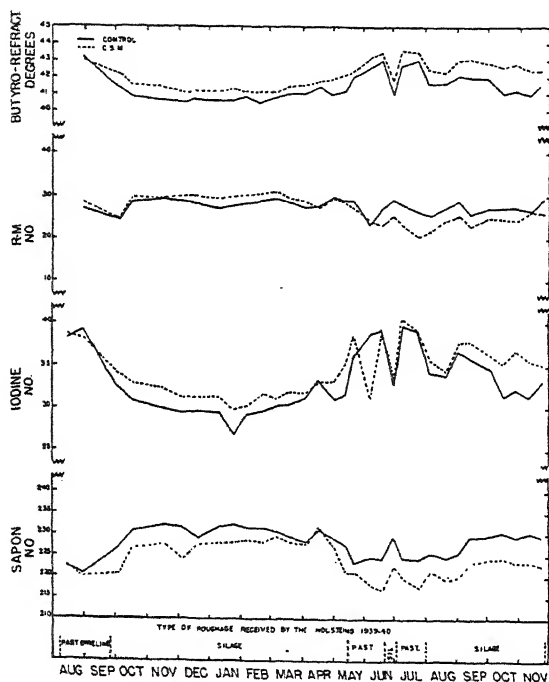


FIG. 1. The saponification number, iodine number, Reichert-Meissl number and butyro-refractometer degrees of the milk fat produced by a group of cows receiving cottonseed meal as the only concentrate as compared with the same fat constants of the milk fat produced by a comparable group of cows receiving a normal concentrate mixture.

in the iodine number were variable, which is in harmony with the results presented in the literature. The iodine number is easily influenced not only by the concentrate but also by the type of roughage. The effects of a change in roughage on the iodine number depend upon the concentrate fed; hence, the variable results obtained during these sudden changes in roughage might have been expected.

The Reichert-Meissl numbers obtained show the greatest deviation from the expected. During the first nine months of the feeding trial, these values

for the milk fat produced by the two groups were practically the same. For the remainder of the trial the milk fat from the cottonseed meal group had the lower Reichert-Meissl number as expected. Although the effects of oil feeds are known to be modified by silage and by pasture, neither this nor any other known factor explains adequately the long time required for the cottonseed meal to affect the volatile acids of the milk fat.

The refractive index was the only fat constants that revealed a consistent difference between the milk fats from the two groups of cows. The milk fat from the cottonseed meal group always had a higher refractometer reading than that of the control group. Most of the reports in the literature show a similar increase of the refractive index resulting from the feeding of cottonseed and its products.

TABLE 1

*The stability value and acid degree of milk fat produced by cows receiving cottonseed meal as the only concentrate compared with milk fat from cows receiving a normal grain mixture*

Date sampled	Stability value		Acid degree	
	C.S.M.	Control	C.S.M.	Control
7-24-40	27.5	32.0	0.42	0.56
8- 6-40	34.5	33.5	0.65	1.35
8-20-40	32.0	33.0	0.57	0.60
9- 3-40	32.0	36.0	0.54	0.78
9-17-40	28.5	39.0	0.62	0.72
10- 1-40	26.0	32.0	0.70	0.57
10-15-40	21.0	25.0	0.60	0.58
10-29-40	28.0	35.0	0.58	0.58
11-12-40	18.0	30.0	0.74	0.54
11-26-40	34.0	37.0	0.56	0.43

A number of factors are known to affect the values obtained in the determination of the susceptibility of fat to oxidation. After eliminating the possibility of the usually recognized sources of error, it was found that an additional factor, the presence of free fatty acids, had a greater influence on fat oxidation than anticipated (1). Before the effect of feed could be measured, it was necessary to eliminate the possibility of even slight lipolytic action on the milk fat.

A few comparisons of the stability value (5) and of the acid degree of the milk fat produced by the two groups of cows are presented in table 1. The milk fat from the cottonseed meal group was found to be less stable toward oxidation than that of the control group. This agrees with investigations showing that the stability of filtered fat varies with the degree of unsaturation. No consistent difference was observed in acid degree between the two milk fats. Unpublished preliminary work on the determination of free fatty acids in milk shows that the churning method probably is not a reliable procedure for obtaining fat for use in measuring the free fatty

acids in milk. Thus the acid degrees presented herein may or may not represent the true relative values.

#### SUMMARY

In general the feeding of cottonseed meal as the only concentrate as compared with feeding a normal grain mixture decreased the saponification number and increased the iodine number and the refractive index. The expected decrease in Reichert-Meissl number did not become evident until after nine months of the feeding period had passed.

In a similar comparison, the milk fat from the cottonseed meal group was less stable toward oxidation than that from the control group. No difference in acid degree of the two milk fats was detected.

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# THE CORRELATION BETWEEN SOME CHARACTERISTICS OF DAIRY BULL SEMEN AND CONCEPTION RATE\*

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The accurate evaluation of freshly drawn and stored bull semen before use is of prime importance in artificial insemination. The detection before use and discarding of semen samples likely to result in poor conception rates is most important in securing maximum efficiency. The physical and chemical characteristics which have been commonly proposed for the evaluation of semen are pH, concentration, motility, viability in storage, percentage of abnormal sperm, glycolysis rate, respiration rate, reduction tests, resistance to various shock treatments, percentage of live sperm, etc. This paper reports a study of the correlation between the first five of the characteristics listed above and the conception rate of the semen as used in the University of Missouri Dairy Herd.

## MATERIALS AND METHODS

The semen studied was largely from University of Missouri bulls of the Holstein-Friesian, Guernsey and Jersey breeds. Cows inseminated were nearly all in the University of Missouri herd, but a few cows and bulls in outside herds have been included in the data. Semen from twenty-three bulls was studied. The bulls studied represented all phases of fertility from practically sterile to highly fertile. The bulls ranged from fifteen months to thirteen years of age.

All inseminations were made by cervical deposition by the use of a speculum as described by Herman and Ragsdale (4). Pregnancy determinations have been supported in most cases by calvings, and in others by manual examinations for presence of fetus or failure of cow to return to heat within 90 days after insemination. The data cover the years 1940 to 1942 and the early part of 1943. Insemination of cows which never conceived or were known to have malfunction of the ovaries or other reproductive trouble were omitted from the study.

The methods used in examination of the semen have been described previously (5). These were simple tests which could be done satisfactorily with little technical skill. The rating of motility from 0 to 5 lacks somewhat in precision, and further study of accurately counting the motile sperm shows that a small number of very active spermatozoa may make the semen appear as good in motility as a larger number of slowly moving sperm.

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Since the vigor of motion as well as the amount is an important test of fertility (2, 7), the rating method may be of great value in spite of its error in estimating the actual number of sperm which are motile. Actual counts have shown that the previously estimated percentage of motile sperm for the different ratings was too high. Semen of 5 motility has about 80 per cent or more progressively motile sperm; 4 motility, 70 to 85 per cent; 3 motility, 45 to 75 per cent; 2 motility, 20 to 50 per cent; and 1 motility is usually below 25 per cent of progressively motile sperm.

Since two or more cows were seldom inseminated with any one sample of semen, the data were grouped to facilitate correlation analysis. All inseminations were tabulated in class interval groups of the semen characteristic as to whether or not they resulted in conception, and the percentage of samples resulting in conception was calculated for each group. The coefficients of linear correlation,  $r$ , were then determined for the various semen characteristics and conception rate.

#### RESULTS

The results secured are presented graphically in figure 1 for all of the characteristics studied except pH. The importance of pH readings was in doubt early in the period covered, hence the pH of all samples was not determined as a routine procedure. The pH of semen used for 205 inseminations was recorded, however. These were grouped in classes of 0.2 pH unit interval from pH 5.8 to 7.4. The correlation coefficient,  $r$ , for pH and conception rate was  $-0.18$  which indicated that there certainly was not a correlation between the two.

The percentage of abnormally shaped sperm in semen used in 525 inseminations was tabulated in groups at intervals of 3 per cent from 0 to 30 per cent. As shown by the chart (fig. 1) and the correlation coefficient of  $-0.12$  there was no correlation between conception rate and percentage of abnormal spermatozoa in the range studied. Bulls producing extremely large numbers of abnormally shaped sperm, however, may be low in fertility (5).

The concentration of sperm in semen used in 559 inseminations was tabulated in groups at intervals of 200 per mm.<sup>3</sup> from 200 to 2000. As shown in figure 1 there was a slight tendency for the more concentrated semen to produce a larger percentage of conceptions. The correlation coefficient, 0.63, was just short of significance, since an  $r$  of 0.666 is required (3) for the expression of a significant correlation within a probability of 0.05. The concentration of sperm was, therefore, not an important factor in the determination of conception rate.

Semen used in 475 inseminations was tabulated as to the length of time a motility rating as good as 2 was maintained in the undiluted semen during storage at 40° F. Daily motility ratings of the stored semen were made; so the samples were grouped at intervals of 24 hours up to 192 hours. Forty-one per cent of inseminations from semen that kept a motility rating of 2 for

less than one day after use produced conceptions. As viability of the semen increased the conception rate increased until 68 per cent of inseminations with the most viable semen group resulted in conception. The correlation coefficient, 0.84, showed that there was a highly significant linear correlation between viability of the sperm in storage and their ability to produce pregnancy.

The motility ratings of semen used in 565 inseminations were tabulated according to the fertility of the semen. As shown in figure 1, plotting the motility ratings against conception rate did not indicate a linear correlation.

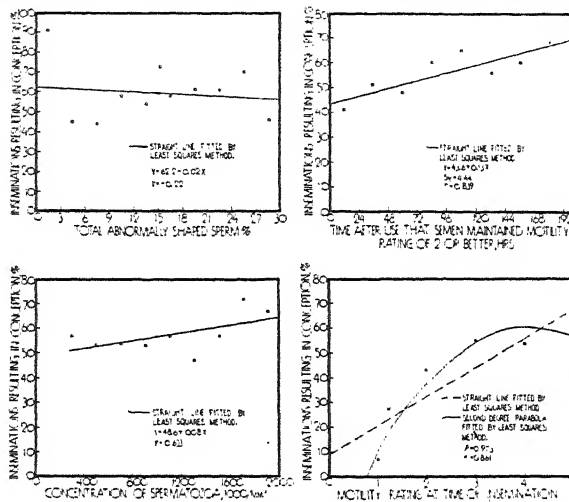


FIG. 1. The correlation between conception rate and semen quality as expressed by motility estimation, viability, concentration of sperm, and abnormal morphology of sperm.

Conception rate from semen of 1 motility was very poor. Semen that was 2 motility was not very satisfactory since only 43 per cent of such inseminations resulted in conception. Semen of 3 motility was correspondingly better than semen of 2 motility as 2 had been better than 1. Conception rate of semen which was rated 4 and 5, however, was not significantly different from that of semen rated only 3, although semen rated 5 motility did give the best conception rate. The relation between motility rating and conception rate was therefore curvilinear. Conception rate increased as motility increased up to the 3 rating, and thereafter increased motility did not result in important increases in conception rate. The index of correlation,  $r$ , should therefore be used to measure the degree of correlation between the two. The index of correlation, 0.97, revealed that there was a significant curvilinear correlation between motility rating and conception rate.

#### DISCUSSION

This evaluation of five commonly used methods of determining semen quality has revealed some useful information for the practitioner. Accord-

ing to the results of this study there is little practical value in making routine pH determinations, abnormality counts, or determining the concentration of sperm. The information furnished by these determinations was not correlated significantly with rate of conception. Therefore, the rejection of a sample of semen because it is slightly abnormal in any of the three characteristics does not seem justified. The only reason for rejecting samples which are very high in pH or abnormalities or very low in concentration should be the extent to which such items are correlated with longevity and motility of the sperm. This problem has not been treated in this study. If samples are of acceptable motility and viability, it does not seem reasonable to reject them on the basis of pH and concentration, especially in view of the fact that the semen may be diluted many times with a buffered protective mixture. Semen that is very high in abnormal sperm usually does not have good motility or viability; but if it does have good motility and viability it will probably give good breeding efficiency.

The fine correlations shown between viability and conception rate are of more theoretical interest than practical interest because such information is not obtained until after use of the semen. This correlation can be used as a guide, however, in the development of other methods of semen evaluation. The high correlation of any test with viability should indicate its correlation with semen fertility. Beck and Salisbury (1) have used short-time high-temperature survival tests in this manner. Knowledge of the probable survival time of highly motile semen is important for selection of semen which must be stored as well as for the maintenance of good conception rates.

The curvilinear relationship between conception rate and motility rating of the semen at the time of insemination will be of special interest in the use of stored semen. Semen which is below a rating of 3 cannot be used with much confidence. Even though many pregnancies have resulted from semen of lower motility the chances of conception are so reduced that such practice may be worthwhile only in the case of very valuable sires. The data also indicate that practically as good results may be expected from semen of 3 and 4 motility as from the highest rating. These observations are in agreement with those of Lasley (6) who found no correlation between motility rating above 3 and the fertility of the semen. He also observed that there was not a significant difference in the fertility of semen containing from 55 to 95 per cent live sperm, but semen which contained only 20 per cent of live sperm was infertile. It therefore seems safe to conclude that semen should be 3 motility or better if good breeding efficiency is to be maintained. In terms of progressively motile spermatozoa, there should be about 45 per cent or more. This study has again demonstrated the value of the motility rating for detecting semen likely to result in poor breeding efficiency. Further study of the value of accurately determining the percentage of progressively motile sperm with reference to its correlation with viability is in progress.



## SUMMARY

A study has been made of the correlation between semen characteristics and conception rate in the University of Missouri dairy herd. The correlation between conception rate and pH, abnormal sperm, and concentration of sperm was found to be non-significant. A highly significant linear correlation was found between conception rate and viability of the sperm. A significant curvilinear relationship between motility and conception rate was found. The difference in conception rate between semen rated 3 motility, which is usually 45 per cent or more progressively motile sperm, and higher grades of motility was very slight.

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## SEASONAL VARIATION IN SEMEN QUALITY OF SOME MISSOURI DAIRY BULLS\*

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The seasonal nature of sexual activity of many mammals is well recognized, but whether or not the bull has a definite seasonal sexual cycle has not been clearly demonstrated. Morgan and Davis (8) found that the conception rate of Nebraska dairy cattle was lowest in August and September, but months with high conception rate were not seasonal since January and July were both high and December was highest in conception rate. Erb, Wilbur and Hilton (5) reported a seasonal variation in breeding efficiency with spring (May at maximum) giving the best conception rate and late summer (August at minimum) giving the lowest conception rate. The separate effect of the bull and the cow upon fertility are confounded in ordinary analysis of breeding records, however.

Few studies have been made of seasonal variations in semen quality. Weatherby, Reece and Bartlett (11) reported monthly averages of semen volume, concentration, and longevity for six bulls in a New Jersey cooperative. Longevity, which is correlated with fertility (10), was generally higher in June and July than in other months, but the differences among the months were very slight. Anderson (1) presented monthly average semen volume and motility observations made on bulls in Kenya which indicated a rather distinct seasonal tendency. Volume and motility were both low from May to August. Erb, Andrews and Hilton (4) made a systematic study of the effect of season upon semen characteristics. Except for a noticeable increase in concentration in the spring, seasons other than summer had little effect upon semen quality. During July, August, and September the motility and semen volume were lowered, the sperm survived for a shorter period, and the percentage of abnormal forms was increased. Statistical analysis showed that semen obtained during the summer months was significantly of lower quality than that of other seasons. Lasley (7) found that beef bulls in Arizona gave higher quality semen as the season progressed from May to September. Volume of semen, percentage of live sperm, and percentage of resistant sperm increased during this period but concentration and percentage of abnormal sperm did not change.

The literature in general is not in complete agreement concerning the effects of season upon semen quality of bulls. The explanation of this fact may be that the effect of season is not as great in some localities as in others.

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In many cases the bulls studied were few in number and their individual responses to season (4) may not have been typical of the population. Since January, 1940, semen of a number of bulls in the University of Missouri herd has been examined regularly. Data for ten of these bulls extended over all four seasons, some of them for two or more years, and from three others, data from two or more seasons were available. This report presents an analysis of the monthly variation in quality of semen produced by these thirteen dairy bulls.

#### MATERIALS AND METHODS

The bulls were all purebred animals used as herd sires in the University of Missouri herd. Nine were Holstein-Friesians, three were Jerseys, and one was a Guernsey. They varied in age from one and one-half to thirteen years, but the majority of them were aged bulls, five year old or older. The average age was six years. Although these bulls were not all of high fertility, none was of consistently very low fertility.

During 1940, semen was collected from each bull at least once each week. Thereafter, however, collections were made only as they were needed for breeding or for various investigational purposes. All bulls were not represented, therefore, in every month of every year included in the study.

Each sample of semen was examined for initial motility, concentration, volume, number of abnormal sperm, and viability according to methods described previously (6). The pH of many of the semen samples was also determined (6).

The observations made on the semen from each bull were averaged by months, and the monthly averages for the separate bulls were then averaged to secure the grand average for that month. If data were available for a certain bull for two or more years for any month, each year's data were handled as a separate monthly average. Statistical significance of the differences among months and among bulls was determined by the analysis of variance according to Snedecor (9).

The bulls were fed a good quality legume hay and a grain mixture the year around. No pasture or green feed was given and no silage was fed. There was no seasonal difference in the rations.

#### RESULTS

Observations on 1103 ejaculates were included in this study. These were separated into 254 bull-month averages, resulting in an average of 4.34 ejaculates per month per bull. The largest number of ejaculates taken from one bull in any month was 12. Many bulls were collected from only once in certain months.

The monthly averages are presented in table 1. The number of bull-month averages from which the monthly average was computed and the

number of bulls represented are listed below the average of each month. The monthly averages are also presented graphically in figure 1.

The seasonal averages are presented in table 1. Winter was taken as January, February, and March. Spring months were April, May, and June. July, August and September were taken as summer; and October, November and December were designated fall months.

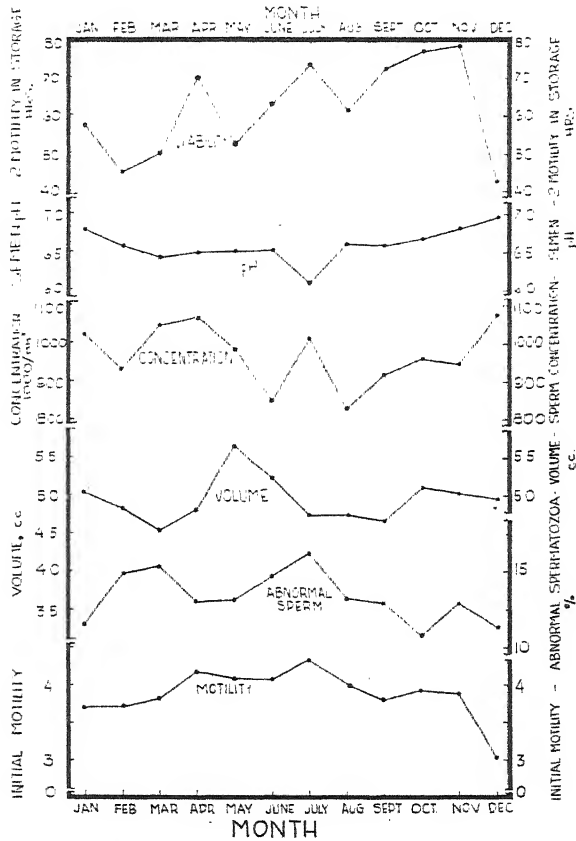


FIG. 1. Monthly averages of examinations for quality of semen from 13 Missouri bulls during 1940, 1941, and 1942.

The data in table 1 and the curves in figure 1 do not indicate very definite seasonal variations of some semen characteristics, but there is a suggestion that others may vary with the seasons. Volume appears to be greater in spring months, initial motility and viability are low in winter months, and pH is lower in summer than in winter. An examination of the original data revealed marked variation among bulls for certain characters in addition to variations among months by the same bull. Furthermore, all bulls did not

exhibit the same sort of seasonal variation. The nature of the data did not permit an accurate assignment of all of the sources of variation by the

TABLE 1  
*Monthly and seasonal summary of semen qualities*

		Volume	Concentration	pH	Initial motility	Maintenance of 2 motility	Abnormal spermatozoa
		cc.	1000/mm. <sup>3</sup>			hrs.	%
January	Mean	5.04	1023.0	6.79	3.71	57.4	11.43
	No.	27	27	17	27	26	26
	Bulls	13	13	11	13	13	12
February	Mean	4.83	931.5	6.57	3.71	44.7	14.82
	No.	24	24	13	24	21	20
	Bulls	11	11	8	11	11	11
March	Mean	4.53	1046.1	6.42	3.81	49.3	15.29
	No.	24	24	10	24	23	20
	Bulls	12	12	8	12	12	12
April	Mean	4.80	1066.7	6.49	4.18	69.8	12.96
	No.	21	21	14	21	21	21
	Bulls	12	12	10	12	12	12
May	Mean	5.65	984.6	6.50	4.09	52.1	13.08
	No.	21	21	12	21	19	21
	Bulls	11	11	8	11	11	11
June	Mean	5.21	851.3	6.52	4.07	62.9	14.65
	No.	20	20	15	20	18	20
	Bulls	12	12	10	12	12	12
July	Mean	4.73	1015.9	6.09	4.33	73.8	16.21
	No.	18	19	7	19	19	18
	Bulls	11	11	7	11	11	11
August	Mean	4.75	831.3	6.60	4.02	61.5	13.25
	No.	17	15	7	17	14	15
	Bulls	10	10	7	10	10	10
September	Mean	4.67	917.9	6.58	3.81	72.1	12.93
	No.	21	21	7	21	21	21
	Bulls	11	11	7	11	11	11
October	Mean	5.12	961.0	6.68	3.94	77.2	10.75
	No.	21	21	11	21	21	21
	Bulls	11	11	9	11	11	11
November	Mean	5.04	944.7	6.81	3.90	78.8	12.96
	No.	18	19	13	19	18	18
	Bulls	11	11	9	11	10	11
December	Mean	4.98	1075.1	6.96	3.07	42.7	11.47
	No.	19	20	7	20	19	20
	Bulls	9	10	6	10	9	10
Year	Mean	4.95	975.7	6.60	3.88	61.6	13.23
	No.	251	252	133	254	240	241
	Bulls	13	13	13	13	13	13
Winter	Mean	4.80	1000.2	6.59	3.74	50.5	13.85
Spring	Mean	5.22	967.5	6.50	4.11	61.6	13.56
Summer	Mean	4.72	921.7	6.42	4.05	69.1	14.13
Fall	Mean	5.05	993.6	6.82	3.64	66.2	11.73

analysis of variance method (9). Variation among months and variation among bulls, however, were calculated and their significance was determined.

*Volume.* The differences in volume of ejaculates among bulls were highly significant. This finding was to be expected because bulls differ widely in this respect. The inter-monthly volume variations were not significant, however; hence a significant seasonal variation of semen volume was not found. Differences as great as were observed could have occurred at least 1 in 20 times as a result of chance. Thus, although the data are suggestive of a greater volume of ejaculate produced in spring than summer, the average difference of 0.5 cc. could easily have occurred as a result of chance.

*Concentration.* The differences in sperm concentration among bulls were highly significant. The differences among months, however, were not significant since they could be expected as a result of chance slightly more than 1 in 20 times. The same was true of the inter-seasonal differences. The concentration of sperm was, therefore, not significantly lower in summer than in winter.

*pH.* The pH differences among months were significant statistically. At  $p = 0.05$  July's average was significantly lower than all other months, and December's average was significantly higher than all months except January, October, and November. The difference between the summer average and the fall average was highly significant ( $p = < 0.01$ ). The pH of fall and winter semen seemed definitely higher than the pH of summer semen, but most of the difference was due to the very low values secured in July.

*Initial motility.* The differences in initial motility among the different bulls were highly significant. The differences among months were also highly significant. Using  $p = 0.01$  as the level of significance the initial motility of July semen was significantly higher than that of December, January and February; and December was significantly below all other months. Using the lower level,  $p = 0.05$ , as a level of significance, April initial motility was significantly above that of December, January and February; July was significantly better than November, December, January, February, March and September; and December was significantly below all other months. Grouping the months into seasons (table 1) showed that spring and summer semen had a significantly higher motility than fall and winter semen. The difference between fall and winter semen was not significant, and spring and summer semen did not differ significantly in initial motility. The semen collected during the warm months definitely had more vigorous motility than that collected during the cold months of fall and winter.

*Maintenance of useful motility in storage.* The storing ability of the undiluted semen showed significant difference among the bulls and among the months. With  $p = 0.01$  representing the level of significance, the storage time of December was significantly below July, September, October, and November; July, October, and November were significantly above February;

and, October and November were significantly above March. When  $p = 0.05$  was taken as the significant point, December and February storage was significantly below that of April, July, September, October and November; and February, March, May and December were significantly below October and November. When the months were grouped into seasons, the average storage time of the winter months was significantly lower than that of the summer and fall months, but the differences between winter and spring months may have occurred as a result of chance 1 out of 20 times. The storing ability of the semen was markedly depressed by the cold months, December to March, and it improved in general after that time to reach its peak in late summer and early fall.

*Abnormal spermatozoa.* The differences in abnormal sperm production among bulls were highly significant. The variation among the bulls was so great that the small differences among the months were not significant. The greatest percentage of abnormal sperm was produced in summer and the least in the fall. Grouping the months into seasons made the difference between summer and fall on the borderline of significance ( $p = 0.05$ ). The other seasons did not differ significantly from each other in any combination, hence the importance of the difference observed between morphology of summer and fall semen is considered to be very slight.

#### DISCUSSION

The most significant seasonal change in the average semen quality of the bulls studied was a decrease in initial motility and viability and an increase in pH with the onset of cold weather in December. These indications of poorer quality semen were probably due to the poorer physical vigor or inadequate sexual stimulation of the old bulls in cold weather. These changes were confined almost entirely to the bulls which were over four years old. The semen from young bulls was as good in motility and viability in December and January as it was in June and July. Since the ejaculates from young bulls were a small minority in this study, however, the averages exhibit more nearly the picture presented by older bulls. Erb *et al.* (4) also have noted the deleterious effect of very cold weather upon semen quality. Since proved bulls which are to be used widely in artificial inseminating work will usually be fully as aged as the bulls used in this study, the results secured should be borne in mind in caring for them. The breeding chute and collection stall at the University of Missouri are out in the open, unprotected from the elements. The bull sheds are three-sided structures, open to the east. The old bulls were noticeably less vigorous and less eager to serve during cold weather than during warm weather and were somewhat "crampy" in winter. It is probable that greater attention to the comfort of the aged sires would result in improved quality of the winter semen.

The drop in semen quality in summer observed by Erb *et al.* (4) and Anderson (1) did not occur. The heat of summer apparently did not ad-



versely affect spermatogenesis in these bulls. In fact, excellent quality semen was obtained regularly from all bulls during the warmest season. These results concur with the observations of Weatherby *et al.* (11) and Lasley (7). The only indication of a poorer quality of sperm produced in summer was the increased abnormal forms produced in July. Since the percentage of abnormal sperm decreased during August and September, also hot months, it does not seem logical to correlate the increase observed in July with temperature.

It was also observed, in confirmation of Erb's (4) observations, that the volume of the ejaculate and the concentration of spermatozoa were slightly lower in summer than in other seasons. The differences were not statistically significant, however; and it is believed that the decreases were not an indication of lowered spermatogenesis or fertility. Motility and viability of the spermatozoa which are closely correlated with fertility, were above average in the summer months.

The low pH for July was due to a few early determinations in that month which were made on semen which had not been cooled promptly. The air temperature was so high that the metabolism of the sperm was kept at a high rate with a resulting high acid production. It is believed that a larger body of data secured under as uniform controlled conditions as those for August and the following months would not show pronounced monthly variations in semen pH. Anderson (2) did not find a significant monthly variation in the pH of bull semen.

Considering altogether the six characteristics of bull semen which have been discussed, it does not seem that spermatogenesis in the dairy bull is significantly affected by season. Furthermore, in the young, vigorous bulls under observation no important seasonal effects were observed. The aged bulls seemed to suffer more from cold weather than the younger bulls and showed less sexual drive or vigor in winter. The general lowered physical vigor possibly was reflected in decreased vitality of their spermatozoa. It seems probable, therefore, that season as such may have no important effect upon the semen quality of dairy bulls in Missouri. Environmental conditions that may adversely affect the physical comfort or even the vitality or health of the bull, however, may result in the production of poorer quality semen. The effect of the observed seasonal differences in semen quality upon fertility of bulls used for natural service or for artificial insemination with non-stored semen would probably be insignificant because of the slight difference in conception rate which has been demonstrated between medium and high quality semen (10). Dawson (3) found no significant seasonal differences in the fertility of aged sires from widely distributed stations in the United States used for natural service. Where artificial insemination practices requiring regular collection and storage of high quality semen are followed, the effect of extreme weather conditions

or other factors adversely affecting the physical vigor of old bulls may be responsible for varying conception rates.

#### SUMMARY

A study of the monthly variation of the initial motility, volume, concentration, useful storage time, pH, and morphology of the semen of thirteen dairy bulls used in the University of Missouri herd during three years has been presented. The monthly variations in volume, concentration, and percentage of total abnormal spermatozoa were not statistically significant. The pH of the semen was significantly lower in the summer than in the fall. Initial motility and useful viability were lower in winter than in spring and summer. The results were interpreted as being largely due to the adverse effect of winter weather upon the physical well being and sexual activity of the aged bulls which furnished the majority of the semen studied.

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## WHEAT GERM OIL AS AN ANTIOXIDANT IN DAIRY PRODUCTS

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The use of antioxidants in the manufacture of food stuffs containing oils and fats has been studied by a number of investigators. Greenbank and Holm (13) found maleic acid, hydroquinone, and certain other phenolic compounds to have antioxidant properties. Chilson (3), Dahle and Palmer (10), and Greenbank (12) reported that the addition of 20 to 100 milligrams of pure vitamin C per liter of milk retarded or entirely prevented the development of an oxidized flavor in susceptible milk to which no copper had been added. Barnicoat and Palmer (2) studied the antioxidant properties of vitamins A, B, C, D, E, F, and nicotinic acid and found that only vitamins C and E were effective.

György and Tomarelli (14) found corn, oats, wheat, rice, bran extract and Avenex to have high antioxidant properties. Of the known B vitamins, p-aminobenzoic acid was the only one they found to be significantly antioxygenic.

Tracy and Corbett (24) have shown that the addition of 0.1 to 0.25 per cent sodium citrate retarded the development of oxidized flavor in milk. Anderson (1) found that a pancreatic extract when added to milk protected it from developing an oxidized flavor.

The work of Dahle and Josephson (6, 7, 8), Corbett and Tracy (4), Koenig (17), Maack and Tracy (18), Mueller and Mack (20), and Peters and Musher (23) has shown that the use of oat flour or a water extract from the oat flour prevented or retarded the oxidized flavor development in milk, ice cream, frozen cream, and butter. Mueller and Mack (21) in studying the antioxidative properties of several cereal flours found that whole oat flour, finely milled oat flour, and corn flour were of equal value but that wheat, barley, rye, and rice flours had but little antioxidative effect.

Corbett and Tracy (5) reported the effectiveness of tyrosine, tyrosine esters and the water extract of certain cereal flours as antioxidants in dairy products. Hollender and Tracy (15) experimented with the use of certain antioxidants in powdered whole milk. Among the antioxidants they found most effective were gum guaiac, hydroquinone, ascorbic acid and sodium citrate.

The studies of McFarlane of MacDonald College, Quebec, as reported by correspondence with the author, have shown wheat germ oil to have antioxi-

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dant properties when added to certain animal and vegetable fats and oils used in the manufacture of shortening. The results were measured by means of the Swift's stability test. This investigator also found wheat germ oil particularly when fortified with citric acid retarded oxidative changes in powdered whole milk as measured by the peroxide value. McFarlane finds wheat germ oil to be more effective than either hydroquinone or gum guaiac.

Realizing the importance of copper contamination in the development of the oxidized flavor, modern dairies use stainless steel in the construction of their equipment wherever possible. In spite of this and other precautions that are taken to retain a normal flavor in the stored products, the industry still recognizes oxidized flavor development in dairy products as a serious problem. This is particularly true in the case of powdered whole milk, much of which is likely to be stored for some time at high temperatures (70° - 125° F.) before consumption. Because of the important part that powdered whole milk plays in the dietary of our armed forces as well as that of the civilian population of our allies, it seems that serious consideration should be given to the application of all known methods that will lengthen the shelf life of the product. Since any oxidation process to which the fat is subjected is likely to result in the destruction of certain of the vitamins contained in milk (Pavcek and Shull (22)), it would also seem desirable from a nutritional point of view to add harmless antioxidants to those dairy products that are to be stored for some time before consumption.

#### EXPERIMENTAL DATA

The use of solvent extracted wheat germ oil as an antioxidant in milk, frozen cream, and powdered whole milk made by both the spray and roller methods has been studied. Representative results are given.

*Use of wheat germ oil in pasteurized milk.* An attempt was first made to determine to what extent wheat germ oil would retard the development of an oxidized flavor in unhomogenized whole milk. The wheat germ oil was added at three levels, 0.1, 0.2, and 0.3 per cent of the weight of the fat. The oil was mixed with the cold milk before pasteurization (143.5° F., 30 min.). The milk used was produced in late spring (May). To a portion of the milk, copper was added at the rate of 1 ppm. and 2 ppm. After 24 hours storage at 40° F. the samples were judged for flavor. The results are given in table 1.

A definite retardation of oxidation was evident in those samples containing the wheat germ oil and to which copper had been added. No particular benefits were obtained at levels higher than 0.2 per cent of the wheat germ oil. The best flavor was in the samples containing no added copper to which 0.1 per cent of wheat germ oil was added. At the 0.2 and 0.3 per cent level enough of the wheat germ oil flavor was noticeable to cause the milk to score less than the control.

TABLE 1

*Wheat germ oil (A) as an antioxidant in whole milk*

Sample	Flavor score* after 3 days' storage at 40° F.	
	Score	Criticism
Control .....	36	Sl. bitter
Control + 0.1% W.G.O. ....	37	Sl. oil flavor
Control + 0.2% W.G.O. ....	35	Sl. oil flavor
Control + 0.3% W.G.O. ....	34	Sl. oil flavor
Control + 1 ppm. Cu .....	29	Oxidized
Control + 1 ppm. Cu + .1% W.G.O. ....	31	Oxidized
Control + 1 ppm. Cu + .2% W.G.O. ....	32	Oxidized
Control + 1 ppm. Cu + .3% W.G.O. ....	32	Oxidized
Control + 2 ppm. Cu .....	28	Oxidized
Control + 2 ppm. Cu + .2% W.G.O. ....	31	Oxidized
Control + 2 ppm. Cu + .3% W.G.O. ....	31	Oxidized

\* Basis of 45 as perfect score.

In other experiments it was found that at levels higher than 0.3 per cent of the weight of the fat the wheat germ oil flavor was sufficiently detectable in the milk to be objectionable even when copper was present.

*Use of wheat germ oil in frozen cream.* To study the effect of wheat germ oil in retarding the development of oxidized flavor in frozen cream, cream containing 50 per cent fat was mixed with sufficient sucrose to produce a sugar content of 12 per cent. Half of this sweetened cream was pasteurized at 160° F. for 30 minutes and the other half at 170° F. for 15 minutes. To half of each of the two lots 0.5 ppm. of copper was added. To a portion

TABLE 2

*Wheat germ oil (A) as an antioxidant in frozen cream*

Sample*	Degree of oxidized flavor after			
	4 mo.	6 mo.	9 mo.	13 mo.
A—Cream pasteurized at 160° F. for 30 minutes				
1A—Control .....	—	—	—	2½+
1B—Control .....	+	+	6+	7+
2A + 0.2% W.G.O. ....	—	—	—	—
2B + 0.2% W.G.O. ....	—	+	2+	4+
3A + 0.3% W.G.O. ....	—	—	—	—
3B + 0.3% W.G.O. ....	—	?	½+	3+
B—Cream pasteurized at 170° F. for 15 minutes				
1A .....	—	—	—	—
1B .....	—	—	+	5+
2A + 0.2% W.G.O. ....	—	—	—	—
2B + 0.2% W.G.O. ....	—	—	½—	2½+
3A + 0.3% W.G.O. ....	—	—	—	—
3B + 0.3% W.G.O. ....	—	—	?	2

\* A = no copper added.

B = ½ ppm. Cu added.

of each of the four lots wheat germ oil at two levels (0.2 and 0.3 per cent of the weight of the fat) was added. The samples were stored in quart Seal-right containers in an ice cream hardening room and were judged every 3 to 4 months over a period of about 13 months. The results given in table 2 show that wheat germ oil caused a definite retardation of the oxidation of butterfat when copper was present. It is also evident that heating cream above 160° F. (170° F.) during pasteurization also aided in retarding oxidation. This confirms the results of Trout and Scheid (25) as well as Dahle, Lawhorn, and Barnhart (9), and McFarland and Burgwald (19). The beneficial effect of the higher heat treatment has been found by Gould and Sommer (11) and Josephson and Doan (16) to be due to the production of sulfhydryls which are reducing substances. Best results were obtained when both the wheat germ oil and the high heat treatment were used. The superior effect of the combination was noticeable, however, only after nine months of storage.

*Wheat germ oil as an antioxidant in powdered whole milk.* Preliminary studies having established the antioxidant properties of wheat germ oil in milk and cream it was desired to determine to what extent the substance would function in powdered whole milk. A complete understanding of the nature of the ingredient contained in wheat germ oil that is responsible for the antioxidant properties is lacking. It is thought, however, that the tocopherols and phosphatides that are present are contributing agents. Since the start of this study it has been found that the potency of wheat germ oil can be increased by the addition of citric acid. The use of sodium citrate as an antioxidant in milk has been previously studied by Tracy and Corbett (24). Three different oils<sup>1</sup> were used, formula A—regular wheat germ oil, formula C—regular wheat germ oil plus 2 per cent citric acid, and formula D—regular wheat germ oil plus 5 per cent citric acid and 30 per cent soybean lecithin.

*Procedure followed in preparing milk powder samples.* Powdered whole milk prepared by both the vacuum roll and spray processes was used to test the antioxidant properties of the wheat germ oil. The milk used for the roller drying was produced by the University herd, while that spray dried was received at a commercial condensery. An attempt was made to handle the milk in such a manner as to minimize copper contamination.

The powder was packed in number 2 cans in the case of the roller process and number 1 cans in the case of the spray drier. Paper containers were used in one of the experiments with spray powder (samples 5019 and 5020—table 4). One set of the spray powders was packed plain and one set was packed in nitrogen.

The milk used to make the powder manufactured by the roller method (table 3) was preheated at 170° F. for 30 minutes and concentrated to ap-

<sup>1</sup> The wheat germ oil used in this study was solvent extracted and was supplied by the VioBin Corporation, Monticello, Illinois.

proximately 38 per cent total solids. Wheat germ oil—formulas A, C, and D, were added to the condensed milk at the rate of 0.2 per cent of the weight of the fat in trial 1 and 0.1 per cent in trial 2.

In the case of the spray dried samples 4924-31 and 4952-59 (table 4) the wheat germ oil was added to skim milk that had been heated to 170° F. and homogenized at 2,500 pounds pressure. This emulsion was then added to the raw milk and held over night. The milk was preheated at 180° F. for 7 minutes, cooled to 145° F. and then condensed. It was homogenized at 3,000 pounds at 130° F. The drying was then done 24 hours later. The nitrogen-packed powder was first subjected to a vacuum of 2 mm. Then CO<sub>2</sub> was introduced to 10 pounds pressure for 10 minutes, then a vacuum of 2 mm. was drawn, then nitrogen was introduced to a pressure of 5 pounds for 10 minutes.

TABLE 3

*Use of wheat germ oil in roller (vacuum) process powdered whole milk*

Sample	Trial	Flavor score		
		Fresh	3 mo.	6 mo.
Control .....	1*	38	29	29
Control + 0.2% W.G.O. A .....	1	38	33	32
Control + 0.2% W.G.O. C .....	1	38	34	32
Control + 0.2% W.G.O. D .....	1	38	34	33
Control .....	2†	38	37	33
Control + 0.1% W.G.O. A .....	2	38	36	35
Control + 0.1% W.G.O. C .....	2	38	37	35
Control + 0.1% W.G.O. D .....	2	38	37	36

\* Prepared Nov., 1942.

† Prepared Feb., 1943.

Samples 4887-90 and 5017-22 were prepared in the same manner as those above except that nitrogen only was used in the case of the gas-treated lot.

The only variation in the procedure followed in the preparation of samples 4985-88 from that followed in the preparation of sample 4887-90 was the method of adding the wheat germ oil. Formula D was used in all samples. In the case of samples 4985 and 4986 the oil was homogenized in skim milk and added to the whole milk before condensing as outlined above. In the case of samples 4987 and 4988 the oil was mixed with the condensed milk just before spraying.

The dried milk samples were judged when fresh and every 2 to 3 months during storage at room temperature. In table 4 only the final flavor scores are reported. The flavor of the reconstituted samples was 38-39 at the beginning of the experiment and was criticized as being cooked. Subsequent scorings were made on the basis of the degree of oxidized flavor developed. The samples were reconstituted by mixing 30 grams of the powder with 200 ml. of tap water using an electric malted milk machine. The sample cans were opened immediately before the milk was reconstituted.

TABLE 4  
Use of wheat germ oil in spray dried whole milk powder

Vis. sample	No.	Date made	Preheat temp. before condensing	Conc. of solids	Spray temp.	Spray pressure	Spray chamber temp.	Temp. of powder when packed	Type of pack*	Sol. in dex	H <sub>2</sub> O	Wheat germ oil used	Gas content†		Flavor score Aug. 7, 1943
													CO <sub>2</sub>	O <sub>2</sub>	
4924	11/24/42	180° F. 7 min., cooled to 145° F.	115	11.42 F.	1,000 lbs.	170° F.	106	° F.	P	cc.	2.1	None	3.64	4.24	30
4925	11/24/42														
4926	11/24/42														
4927	11/24/42														
4928	11/24/42														
4929	11/24/42	180° F. 5 min.	115	11.44 F.	1,800	170	104	° F.	P	cc.	2.1	None	3.16	3.99	29
4930	11/24/42														
4931	11/24/42														
4952	12/ 2/43														
4953	12/ 2/43														
4954	12/ 2/43	170° F. 30 min.	130	11.59 F.	2,500	165	122	° F.	P	cc.	2.0	None	0.25	3.54	23
4955	12/ 2/43														
4956	12/ 2/43														
4957	12/ 2/43														
4958	12/ 2/43														
4959	12/ 2/43	180° F. 5 min., cooled to 145	130	10.90 F.	2,500	170	90	° F.	P	cc.	2.1	0.1% D	0.20	3.20	30
4887	11/10/42														
4888	11/10/42														
4889	11/10/42														
4890	11/10/42														
4985	12/12/42	170° F. 30 min.	130	10.06 F.	800	180	64	° F.	P-tin	cc.	1.8	None	0.28	1.45	32
4986	12/12/42														
4987	12/12/42														
4988	12/12/42														
5017	1/13/43														
5018	1/13/43	170° F. 30 min.	130	10.87 F.	2,500	170	90	° F.	P	cc.	2.8	0.1% D	3.09	4.05	35
5019	1/13/43														
5020	1/13/43														
5021	1/13/43														
5022	1/13/43														

\* P = plain.

N = gas.

† Lots 5018 and 5021 were packed with water pumped nitrogen, while the other lots were packed with oil pumped nitrogen.



Other details regarding the procedure followed in making the spray powders are given in table 4.

*Milk powder data.* The data obtained on the effect of wheat germ oil upon the keeping quality of powdered whole milk are given in tables 3 and 4. It is evident that wheat germ oil retarded the oxidation of butter fat but did not prevent its occurrence. The oil treated with citric acid was slightly superior to the untreated oil. Formula D gave results somewhat better than those obtained with Formula C in most cases. The method of adding the oil was of no particular importance. The oil functioned in both plain and nitrogen-packed powder. It should be pointed out that neither the use of wheat germ oil or gas packing prevented oxidation from taking place though packing in the presence of an inert gas proved to be somewhat more beneficial than the use of the wheat germ oil without gassing. Best results were obtained with a combination of the two, however.

#### SUMMARY AND CONCLUSIONS

The ability of wheat germ oil to prevent oxidation has been determined in fluid milk, frozen cream and powdered whole milk made by both the vacuum roll and spray processes. The amount of wheat germ oil needed for best results is approximately 0.2 per cent of the weight of the fat. At higher levels (0.3 per cent) the flavor of the oil is sometimes detectable. Wheat germ oil reinforced with citric acid was found to be more effective in retarding oxidation in milk powder than regular oil. While wheat germ oil is not as effective as gas packing with nitrogen in preventing the development of the oxidized flavor in powdered milk, a combination of the two will prolong the shelf life of the powder more than either one alone will accomplish.

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TENTATIVE PROGRAM  
AMERICAN DAIRY SCIENCE ASSOCIATION MEETING

TUESDAY, JUNE 20, 1944

*Morning Session*

9:00-12:00—General Meeting.

Welcome.

Response and presidential address—PRESIDENT A. C. DAHL-  
BERG.

Address by guest speaker.

*Afternoon Session*

1:00- 4:00—Sectional Meetings—Extension Section, Manufacturing Sec-  
tion, and Production Section.

4:00- 5:00—Committee Meetings—Extension Section, Manufacturing  
Section, and Production Section.

*Evening Session*

Production Section Tentative Symposium.

WEDNESDAY, JUNE 21, 1944

*Morning Session*

9:00-11:00—Sectional Meetings—Extension Section, Manufacturing Sec-  
tion, and Production Section.

11:00-12:00—Joint Business Meeting of the Production Section and the  
Extension Section.

—Business Meeting of the Manufacturing Section.

*Afternoon Session*

1:00- 4:00—General Session—Post War Problems in Dairying.

Introductory Speaker.

From the Extension View Point.

From the Production View Point.

From the Manufacturing View Point.

4:00- 5:00—Committee Meetings—Extension Section, Manufacturing  
Section, and Production Section.

THURSDAY, JUNE 22, 1944

*Morning Session*

9:00-11:00—Sectional Meetings.

Manufacturing Section Symposium—Dehydrated Milk  
and Milk Products.

## Joint Symposium—Production and Extension Sections.

9:00–9:15—Mastitis (from the dairyman's standpoint), T. S. SUTTON, Ohio State University.

9:15–9:45—Modern methods of treating mastitis, C. S. BRYAN, Michigan State College.

9:45–10:15—Discussion.

10:15–10:45—The feed situation, MR. WALTER BERGER, Chairman of the Feed and Livestock Division, Food Production Administration.

10:45–11:00—Discussion of feed situation.

11:00–12:00—Sectional Business Meetings—Extension Section, Manufacturing Section and Production Section.

*Afternoon Session*

1:00–3:30—Latin American Dairying.

WILLIAM S. HENDRIX, Ohio State University.

R. E. HODGSON, United States Department of Agriculture.

A. C. DAHLBERG, Cornell University.

3:30 —General Business Meeting.

*Evening Session*

6:30 —Annual Banquet.

# JOURNAL OF DAIRY SCIENCE

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## A STUDY OF CREAM RISING IN MILK

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The creaming process is a complex phenomenon that involves a number of factors. The difference in density between the fat and the milk plasma is the primary factor but a number of secondary factors, concerned with the formation of globule clusters, are required to make normal creaming possible. It has been shown that the individual globules rise in harmony with the velocity computed by Stokes' formula (4, 7, 8, 9), but this is too slow to account for the rapidity of normal creaming, the depth of the cream layer produced, and the sharp line of demarcation between the cream and skim-milk layers. The secondary factors, therefore, exert a controlling effect on the gravitational separation for creaming. The nature of these secondary factors and the detailed manner in which they function is still a matter of controversy.

The theory has been advanced that, aside from other factors, the gravitational movement of the globules (and clusters) is an important factor in cluster formation (1, 2, 5). The larger globules rising more rapidly overtake smaller globules, and as the clustering process proceeds in this manner, the clusters rise at an increasing rate and thus have increased opportunity for sweeping along individual globules as they occur in the upward path of the cluster. A difference in the fat content of the skim-milk at varied levels might well result from such a process. The study herein reported was undertaken to investigate this possibility.

### EXPERIMENTAL

Samples of milk were placed in 1000-cc. graduated cylinders at 40° F. and stored in a refrigerator at that temperature. Six cylinders were used for each trial to provide for examination of a different cylinder at 1, 2, 4, 6, 10 and 24 hours after the cylinders were filled. Five experiments were conducted, one with fresh, raw milk and four with pasteurized milk. The

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milk had a fat content of 3.6 to 3.8 per cent. It was obtained from the mixed milk of about 40 herds as received commercially at the Department of Dairy Industry, University of Wisconsin. After the milk in the cylinders had stood undisturbed at 40° F. for the desired time, skim milk samples were withdrawn at the following points as indicated by the graduated scale: 850, 750, 650, 550, 450, 350, 250, 150, 50 and 10 cc. The sample taken at the 850-cc. level was approximately 30 to 50 cc. below the cream line. The samples were withdrawn in the order as listed with the aid of the special apparatus shown in figure 1.

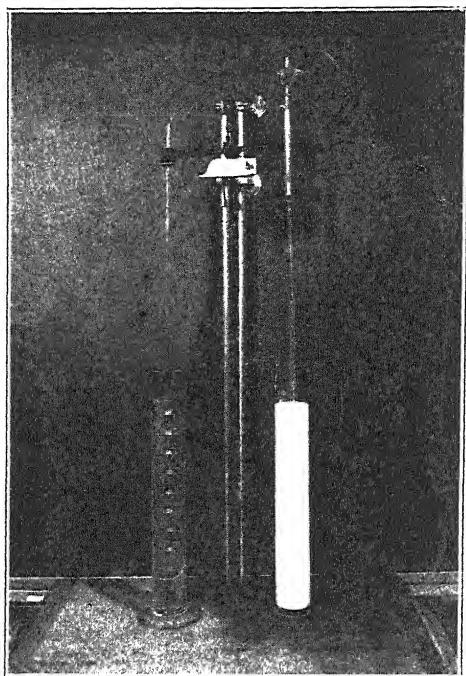


FIG. 1. Sampling device used for withdrawing samples of skim milk at various levels from a graduated cylinder.

In the sampling procedure, entrance of the milk into the pipette was prevented until the tip was at the desired level. Mere closing of the mouth end of the pipette during lowering would not accomplish this since the hydrostatic pressure would compress the air and allow some milk to enter. This was counteracted to a point where a few small bubbles of air would actually escape from the pipette during immersion by simultaneously lowering a tube, connected to the pipette as shown in figure 1, into a sugar solution of sufficient density so that the hydrostatic pressure exceeded that caused by the milk. The assembly also carried a pointer, adjusted to the same level as the

pipette tip, to indicate its position as gauged by the pointer against the outside of the cylinder. When the pipette tip had been immersed to the desired level, the pipette was filled slowly with suitable manipulations of the valves and the application of suction. The entire assembly was then raised, the exterior of the pipette was wiped free from milk, and the sample within the pipette was then discharged into a suitable container. A clean, dry pipette was used for each sample. The fat content of the skimmilk samples was determined by the Mojonnier method as outlined by Mojonnier and Troy (3). All fat analyses were run in duplicate. The duplicate determinations

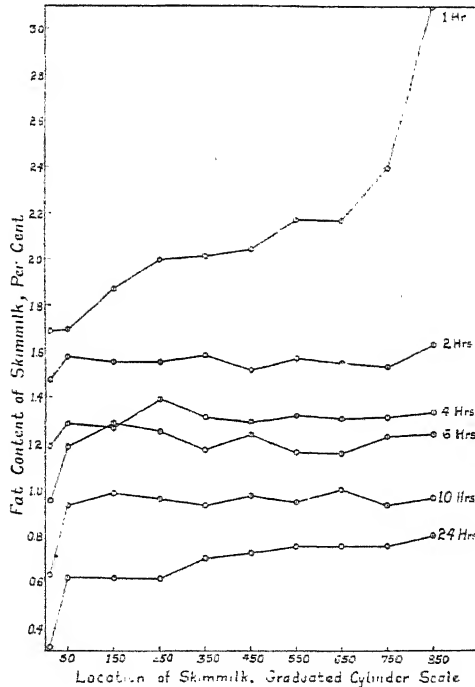


FIG. 2. Fat content of skimmilk at various levels below the cream line in 1000 cc. graduated cylinder used as a creaming vessel for whole pasteurized milk.

were made with considerable care and in general the difference between duplicates was very small in comparison with any observed differences in fat content between the various layers of skimmilk. Tables 1 to 5 give the duplicate fat tests and average fat tests of the skimmilk samples taken at various levels and at creaming times of 1 to 24 hours. The most rapid and extensive fat rising occurred during the first hour with a slow, gradual rise of the residual fat continuing during the succeeding 23 hours. While no data were secured beyond 24 hours, it can be assumed that a slow rising of residual fat would continue indefinitely in harmony with observations of Trelogan and Combs who studied rat rising in cream (6).

TABLE 1

*Fat content of skim milk secured below the cream line in raw milk at varied levels*

Skim milk level	Average and duplicate fat determinations			
	1 hour		2 hours	
		Average		Average
850	2.749		1.218	
	2.743	2.746	1.219	1.218
750	2.222		1.193	
	2.216	2.219	1.205	1.199
650	2.051		1.157	
	2.052	2.051	1.161	1.159
550	1.910		1.179	
	1.910	1.910	1.179	1.179
450	1.783		1.179	
	1.782	1.782	1.176	1.178
350	1.736		1.214	
	1.746	1.741	1.218	1.216
250	1.708		1.243	
	1.711	1.709	1.244	1.244
150	1.603		1.210	
	1.615	1.609	1.210	1.210
50	1.555		1.175	
	1.552	1.554	1.165	1.170
10	1.500		1.118	
	1.499	1.500	1.122	1.120
	4 hours		6 hours	
		Average		Average
850	1.111		0.887	
	1.113	1.112	0.893	0.890
750	1.123		0.906	
	1.108	1.115	0.896	0.901
650	1.125		0.900	
	1.121	1.123	0.901	0.900
550	1.140		0.902	
	1.143	1.142	0.906	0.904
450	1.123		0.900	
	1.117	1.120	0.898	0.899
350	1.129		0.898	
	1.121	1.125	0.896	0.897
250	1.166		0.881	
	1.167	1.166	0.880	0.880
150	1.151		0.896	
	1.149	1.150	0.887	0.892
50	1.136		0.858	
	1.136	1.136	0.865	0.862
10	1.037		0.789	
	1.045	1.041	0.796	0.782



TABLE 2

*Fat content of skimmilk secured below the cream line in pasteurized milk  
at varied levels*

Skim milk level	Average and duplicate fat determinations					
	1 hour		2 hours		4 hours	
		Average		Average		Average
850	3.100		1.627		1.338	
	3.110	3.105	1.631	1.629	1.337	1.338
750	2.397		1.535		1.323	
	2.393	2.395	1.535	1.535	1.303	1.313
650	2.168		1.552		1.307	
	2.170	2.169	1.552	1.552	1.305	1.306
550	2.174		1.572		1.324	
	2.168	2.171	1.561	1.567	1.318	1.321
450	2.044		1.527		1.289	
	2.042	2.043	1.509	1.518	1.289	1.289
350	2.012		1.581		1.312	
	2.012	2.012	1.580	1.580	1.314	1.313
250	1.999		1.543		1.379	
	1.974	1.987	1.555	1.549	1.397	1.388
150	1.873		1.547		1.268	
	1.865	1.869	1.551	1.549	1.263	1.265
50	1.693		1.576		1.282	
	1.690	1.692	1.578	1.577	1.282	1.282
10	1.685		1.472		1.181	
	1.685	1.685	1.479	1.476	1.188	1.184
	6 hours		10 hours		24 hours	
		Average		Average		Average
850	1.240		0.966		0.802	
	1.242	1.241	0.961	0.964	0.801	0.802
750	1.227		0.934		0.757	
	1.227	1.227	0.935	0.935	0.757	0.757
650	1.162		0.997		0.755	
	1.153	1.153	1.001	0.999	0.751	0.753
550	1.161		0.944		0.752	
	1.160	1.160	0.943	0.944	0.754	0.753
450	1.236		0.969		0.730	
	1.237	1.237	0.972	0.970	0.720	0.725
350	1.179		0.932		0.702	
	1.163	1.171	0.930	0.931	0.698	0.700
250	1.252		0.959		0.613	
	1.250	1.251	0.958	0.958	0.613	0.613
150	1.284		0.984		0.613	
	1.283	1.284	0.983	0.984	0.617	0.615
50	1.180		0.931		0.620	
	1.185	1.182	0.932	0.932	0.617	0.618
10	0.956		0.628		0.321	
	0.952	0.954	0.628	0.628	0.307	0.314

TABLE 3

*Fat content of skim milk secured below the cream line in pasteurized milk at varied levels*

Skim milk level	Average and duplicate fat determinations					
	1 hour		2 hours		4 hours	
		Average		Average		Average
850	2.711		1.709		1.368	
	2.705	2.708	1.696	1.703	1.362	1.365
750	2.420		1.696		1.293	
	2.408	2.414	1.697	1.697	1.305	1.299
650	2.325		1.525		1.292	
	2.327	2.326	1.533	1.529	1.280	1.286
550	2.225		1.540		1.277	
	2.215	2.220	1.560	1.550	1.257	1.267
450	1.997		1.515		1.255	
	1.986	1.992	1.524	1.520	1.253	1.254
350	1.969		1.527		1.246	
	1.971	1.970	1.531	1.529	1.240	1.243
250	1.925		1.562		1.327	
	1.925	1.925	1.551	1.557	1.330	1.328
150	1.839		1.531		1.277	
	1.852	1.846	1.531	1.531	1.276	1.277
50	1.823		1.510		1.337	
	1.815	1.819	1.506	1.508	1.322	1.330
10	1.620		1.399		0.994	
	1.615	1.618	1.410	1.404	0.994	0.994
	6 hours		10 hours		24 hours	
		Average		Average		Average
850	1.175		1.058		0.819	
	1.181	1.178	1.059	1.059	0.812	0.816
750	1.132		1.023		0.773	
	1.130	1.131	1.016	1.020	0.773	0.773
650	1.161		1.013		0.763	
	1.161	1.161	1.013	1.013	0.761	0.762
550	1.105		0.964		0.739	
	1.127	1.116	0.974	0.969	0.728	0.734
450	1.108		1.025		0.737	
	1.100	1.104	1.019	1.022	0.733	0.735
350	1.163		1.018		0.752	
	1.161	1.162	1.018	1.018	0.755	0.754
250	1.159		1.004		0.729	
	1.164	1.162	1.002	1.003	0.728	0.728
150	1.165		0.971		0.731	
	1.155	1.160	0.979	0.975	0.739	0.734
50	1.219		0.965		0.716	
	1.211	1.215	0.952	0.958	0.715	0.716
10	0.940		0.907		0.360	
	0.947	0.944	0.906	0.906	0.376	0.368

TABLE 4

*Fat content of skim milk secured below the cream line in pasteurized milk at varied levels*

Skim milk level	Average and duplicate fat determinations					
	1 hour		2 hours		4 hours	
		Average		Average		Average
850	1.825		1.343		1.125	
	1.829	1.827	1.341	1.342	1.128	1.126
750	1.752		1.304		1.114	
	1.740	1.746	1.313	1.308	1.107	1.110
650	1.745		1.321		1.102	
	1.753	1.749	1.315	1.318	1.101	1.102
550	1.715		1.285		1.061	
	1.708	1.712	1.283	1.284	1.070	1.066
450	1.629		1.300		1.011	
	1.646	1.638	1.293	1.296	1.064	1.062
350	1.620		1.318		1.045	
	1.618	1.619	1.331	1.324	1.052	1.048
250	1.360		1.283		1.062	
	1.576	1.568	1.291	1.287	1.069	1.066
150	1.501		1.260		1.077	
	1.499	1.500	1.271	1.266	1.083	1.080
50	1.438		1.233		1.080	
	1.444	1.441	1.236	1.234	1.070	1.075
10	1.347		1.170		0.988	
	1.352	1.350	1.182	1.176	0.981	0.984
	6 hours		10 hours		24 hours	
		Average		Average		Average
850	0.969		0.919		0.654	
	0.965	0.967	0.912	0.916	0.648	0.651
750	0.956		0.906		0.610	
	0.956	0.956	0.909	0.908	0.610	0.610
650	0.942		0.917		0.608	
	0.949	0.946	0.911	0.914	0.614	0.611
550	0.937		0.893		0.589	
	0.942	0.940	0.890	0.892	0.585	0.587
450	0.948		0.820		0.540	
	0.947	0.948	0.819	0.820	0.544	0.542
350	0.930		0.788		0.515	
	0.926	0.928	0.789	0.789	0.525	0.520
250	0.926		0.775		0.545	
	0.928	0.927	0.788	0.782	0.539	0.542
150	0.923		0.777		0.575	
	0.920	0.922	0.767	0.772	0.595	0.580
50	0.922		0.734		0.546	
	0.920	0.921	0.730	0.732	0.544	0.545
10	0.897		0.585		0.408	
	0.894	0.896	0.595	0.590	0.416	0.412

TABLE 5

*Fat content of skimmilk secured below the cream line in pasteurized milk  
at varied levels*

Skim milk level	Average and duplicate fat determinations					
	1 hour		2 hours		4 hours	
		Average		Average		Average
850	1.643		1.341		1.114	
	1.645	1.644	1.346	1.344	1.123	1.118
750	1.561		1.278		1.093	
	1.565	1.563	1.285	1.282	1.103	1.098
650	1.543		1.281		1.150	
	1.537	1.540	1.290	1.286	1.154	1.152
550	1.586		1.272		1.108	
	1.587	1.584	1.286	1.279	1.103	1.106
450	1.575		1.259		1.136	
	1.583	1.579	1.264	1.262	1.142	1.139
350	1.574		1.280		1.103	
	1.578	1.576	1.270	1.275	1.106	1.104
250	1.516		1.281		1.113	
	1.513	1.514	1.283	1.282	1.120	1.116
150	1.488		1.278		1.135	
	1.474	1.481	1.289	1.284	1.132	1.134
50	1.446		1.272		1.112	
	1.452	1.449	1.274	1.273	1.111	1.112
10	1.345		1.237		0.964	
	1.339	1.342	1.237	1.237	0.959	0.962
	6 hours		10 hours		24 hours	
		Average		Average		Average
850	0.950		0.929		0.603	
	0.946	0.948	0.935	0.932	0.602	0.602
750	0.967		0.860		0.657	
	0.963	0.965	0.856	0.858	0.608	0.662
650	0.968		0.855		0.599	
	0.972	0.970	0.862	0.858	0.600	0.600
550	0.964		0.871		0.608	
	0.964	0.964	0.877	0.874	0.618	0.613
450	0.973		0.878		0.621	
	0.971	0.972	0.874	0.876	0.617	0.619
350	0.989		0.867		0.610	
	0.980	0.984	0.874	0.870	0.616	0.613
250	1.006		0.870		0.592	
	1.008	1.007	0.867	0.868	0.604	0.598
150	0.967		0.890		0.587	
	0.960	0.964	0.897	0.894	0.590	0.588
50	0.966		0.865		0.601	
	0.966	0.966	0.861	0.863	0.607	0.604
10	0.870		0.598		0.467	
	0.868	0.869	0.595	0.596	0.462	0.464

In the gravitational theory of clustering, which inspired this study, it had been anticipated that, at some stages in the creaming process, the fat content of the skimmilk at some of the higher levels might actually be less than that of skimmilk at a lower point. This anticipation is on the basis of the greater sweeping action to which the fat globules in the upper portions of the skimmilk are subjected. The data indicate that such differences occur to a slight degree, but surprisingly the data suggest further that there is a wave-like progression of fat in the creaming process. This trend is revealed in the most pronounced manner by the data given in table 2 which have been presented graphically in figure 2.

No explanation is offered for the wave-like progression of the fat in the trials herein reported. While this tendency is suggested, the present approach is not ideal for a complete verification of this point. In order to establish this conclusion definitely, it would obviously be necessary to obtain a large number of points so that each wave in the graph might be defined by several points. It would then be necessary to use a method of sampling which would assure as far as possible a perfect horizontal section of the column of skimmilk at the selected level. While the present equipment was intended to attain a sample of skimmilk at a precise level, its use in sampling at still closer intervals probably would not be justified because of the manner in which the milk flows into the pipette.

#### SUMMARY

It was shown that in the creaming of milk the greater portion of the fat rises during the first hour. A slow, gradual rise of residual fat continues indefinitely. There is some indication in the data that during the creaming process the fat content of the skimmilk at one level may be slightly less than the fat content of the skimmilk at a lower level. Indications were obtained that such differences may occur at several points in a skimmilk column suggesting a wave-like progression of the fat. This suggestion should be further verified before it is accepted as a definite conclusion. There appears to be no difference in the manner in which the cream layer forms on raw milk as compared with pasteurized milk.

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## EFFECT OF ADDING COD LIVER OIL TO THE RATIONS OF DAIRY CALVES\*

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Some calves dropped in the dairy herd at University Farm are weak at birth, and develop indigestion or other ailments and a few die before they are a month old. The cows in the herd are fed largely on a barn-feeding basis throughout the entire year because little or no pasture is available. Fair to good quality roughage is fed. Meigs and Converse (6) have shown that the quality of roughage fed to cows has a marked effect on the health and vigor of their calves at birth. Similarly the beneficial effect of feeding a vitamin A supplement to calves for several weeks after birth or until they are consuming considerable quantities of hay has been demonstrated by Phillips (7). If carotene-poor roughage is fed, or if young calves, for any reason, fail to utilize the carotene supplied then the feeding of a vitamin A-rich product beyond the first few weeks should prove beneficial. Ellenberger (4) reported that neither calves raised to 240 days of age on poor quality roughage nor those fed good hay for the same period evidenced any lack of vitamin A. Calves fed the poor hay, however, made "slower, lesser, and more costly growth" as caused by their lower consumption of nutrients in the less palatable ration. When good quality hay was fed, no benefits appeared from feeding cod liver oil, instead growth in some cases seemed to be retarded. Cary (2) states that calves fed skim milk, grain and hay after 30 to 60 days of age must, in order to survive, receive a vitamin A supplement if fed hay as poor in carotene as U. S. No. 3 alfalfa or timothy. Feeding at the rate of 20 milliliters of cod liver oil per calf daily to six months of age, however, had no demonstrable effect on their general appearance and rate of growth when U. S. No. 2 alfalfa hay or better was fed. Similarly, Insko and Rupel (5) observed no marked difference in the rate of growth and composition of bones between calves fed a standard ration, including good quality legume hay, and others reared on a similar ration supplemented with cod liver oil. Similar results have more recently been reported by Reaves and Cannon (8). In a comparison involving four pairs of calves from dams that presumably obtained a normal allowance of pasture, Dahlberg and Maynard (3) fed varying amounts of Nopco XX, a vitamin A and D rich concentrate of cod liver oil. Both lots weighed exactly the same at the start, but the supplemented group soon forged ahead and at 26 weeks of age each averaged 18.7 pounds more than those in the check group. The

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authors concluded that if there was any difference in general health it was in favor of the calves receiving the supplement.

#### EXPERIMENTAL

The object of the experiment reported here was to determine the effect, if any, on the growth and well-being of dairy heifer calves at University Farm of supplementing their rations with cod liver oil from birth to six

TABLE 1

*Effect on the weight of calves of various breeds of including cod liver oil in their rations to 180 days of age. Data calculated to a 10-day interval basis*

Age	Guernseys		Holsteins		Jerseys	
	Non-cod liver oil	Cod liver oil	Non-cod liver oil	Cod liver oil	Non-cod liver oil	Cod liver oil
	No. in group					
	7	12	14	14	12	13
<i>days</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
Birth	64.0	65.9	94.7	93.6	53.9	53.2
10	66.4	67.2	101.2	97.9	55.8	56.5
20	70.6	72.4	112.7	108.9	62.2	63.3
30	80.5	80.2	126.1	121.8	68.5	72.1
40	86.4	88.3	137.0	138.4	76.8	82.4
50	97.5	94.6	152.6	152.6	90.4	90.0
60	104.4	102.2	171.0	169.6	101.6	101.2
70	114.6	114.4	187.1	185.0	112.6	113.8
80	126.7	127.8	201.8	201.7	122.4	127.2
90	136.1	142.6	219.7	217.3	134.1	141.5
100	149.6	157.4	235.7	235.2	148.5	157.4
110	162.7	171.9	256.0	251.9	159.3	170.2
120	174.9	188.9	274.8	271.8	173.7	186.0
130	189.4	204.5	292.6	290.4	185.5	197.0
140	204.6	222.5	309.5	307.5	201.5	211.2
150	217.2	239.3	329.1	326.2	216.3	226.6
160	235.5	255.1	347.6	350.2	232.9	244.2
170	250.8	270.8	367.3	370.0	247.3	261.1
180	262.5	286.9	389.1	389.0	258.7	275.5
Total gain in 180 days	198.5	221.0	294.4	295.4	204.8	222.3
Average daily gain	1.10	1.23	1.64	1.64	1.14	1.24

months of age. The trials were carried on with all the heifer calves born in the herd during a period of about three years. It included 19 Guernseys, 25 Jerseys and 28 Holsteins. The calves were assigned at birth alternately to the check and experimental groups. The calves in both groups were fed and handled alike except that 25 to 35 cc. of cod liver oil (U.S.P.) was fed daily to each animal in the supplemented group. Whole milk was fed the first 30 days, and then skim milk to six months of age. The milk was fed at the rate of one-eighth the weight of the animal but not over 16 pounds daily per calf. Calves were allowed to eat all the alfalfa hay they wanted



at all times. Fair to good quality hay was fed. A grain mixture consisting of equal parts by weight of oats, corn, and barley (all ground medium fine), and wheat bran was fed *ad libitum* up to four pounds daily per calf. Water and salt was available to the calves at all times. Calves were kept in large roomy pens with four or five of the same breed and of approximately the same age in each. They were allowed to exercise outdoors several hours daily when weather conditions permitted.

Each calf was weighed within a few hours after birth and at regular ten-day intervals thereafter. Measurements of height at withers were made every 30 days beginning within a few weeks after birth. Frequent observations were made of the physical condition and appearance of each calf.

TABLE 2

*Effect on the height at withers of calves of various breeds of including cod liver oil in their rations to 180 days of age. Data calculated to a 30-day interval basis*

Age	Guernseys		Holsteins		Jerseys	
	Non-cod liver oil	Cod liver oil	Non-cod liver oil	Cod liver oil	Non-cod liver oil	Cod liver oil
	No. in group					
	7	12	14	14	12	13
<i>days</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
10	70.4	71.8	74.8	74.1	67.3	67.9
30	72.4	73.5	77.6	78.1	69.4	69.8
60	76.6	76.8	83.4	83.6	75.1	75.0
90	81.2	81.2	89.4	88.8	80.1	80.8
120	86.0	86.6	94.2	94.0	84.8	86.3
150	90.4	91.8	99.2	98.6	89.8	90.5
180	94.7	96.2	104.2	103.1	93.4	95.0
Total gain in height in 170 days	24.3	24.4	29.4	29.0	26.1	27.1
Average daily gain	0.14	0.14	0.17	0.17	0.15	0.16

# RESULTS AND DISCUSSION

Data are presented in table 1, showing the weights at ten-day intervals, from birth to 180 days of age, of groups of calves of the Guernsey, Holstein, and Jersey breeds respectively, when fed cod liver oil and of corresponding non-supplemented groups. Data relating to the average height at withers of the animals in the various groups at 30-day intervals are presented in table 2. As height measurements of calves at birth were seldom made, none are given in the table.

From the data in tables 1 and 2 it is apparent that adding cod liver oil to the rations had no evident effect on the rate of gain in height at withers of calves of any breed nor on the gain in weight of Holsteins. On the other hand the Guernseys and the Jerseys fed cod liver oil, gained an average of

22.5 pounds and 17.5 pounds more respectively per calf during the 180 days than those in corresponding non-supplemented groups. Statistical analysis of the data indicate that these differences are slightly significant (9).

The recorded observations indicate that less trouble from digestive disturbance was encountered in young calves that were fed cod liver oil than among those in the non-supplemented groups. It is also significant that a few calves in the non-cod liver oil groups died within a few weeks after birth, and others apparently were saved from this fate when cod liver oil was added to their rations. These facts largely account for the difference in number of calves in the supplemented and the non-supplemented groups. Data relating to the calves that died during the experimental period or that were transferred to a supplemented ration are not included in tables 1 and 2.

Contrary to what may have been expected, as based on the studies of Phillips (6) the cod liver oil supplement had no apparent effect on the rate of gain of calves until after they were about 90 days old. It seems probable that the whole milk, which, generally is a rich source of vitamin A and carotene, helped to build up a small reserve of these factors in the body of the calf during the first 30 days when it was fed and this together with the carotene obtained in the limited quantity of hay consumed provided sufficient vitamin A for growth to about 90 days of age. Beyond that age, however, with the vitamin A reserve of the body largely exhausted the supply obtained from the roughage in the ration was not fully adequate except in the case of the Holstein calves. This difference may have been due to a higher carotene requirement by the Guernsey and the Jersey calves as has been suggested by Boyer *et al.* (1), or to their inability to utilize this factor as efficiently as the Holsteins. Also, it is possible that the Holstein calves consumed proportionately more roughage and thus obtained correspondingly more carotene. Unfortunately complete records were not kept of the quantity of hay consumed by each calf. To what extent results would have been changed had excellent, instead of fair to good, quality hay been fed can only be surmised. From the data presented it appears that it may be beneficial to feed cod liver oil to young calves, especially Guernseys and Jerseys, when roughage only fair in quality is fed.

#### SUMMARY

Comparisons were made using a total of 72 calves, including 19 Guernseys, 28 Holsteins and 25 Jerseys, to determine the effect, if any, of adding cod liver oil, as a vitamin A supplement to the rations commonly fed to calves during the period to six months of age. Alternate animals were fed 25-35 cc. cod liver oil daily beginning when the calf was only a few days old, in all other respects the plan of feeding was alike for both groups.

Somewhat less digestive troubles occurred in the calves fed cod liver oil than among those in the non-supplemented groups. No significant differ-

ence was noted in any breed in rate of gain in height at withers between the calves fed cod liver oil and those in the check group. Adding cod liver oil to the ration had no evident effect on the rate of gain in weight of Holstein calves. The Guernsey and the Jersey calves fed cod liver oil, on the other hand, gained an average of 22.5 pounds and 17.5 pounds more per calf respectively during the 180-day period than those fed similar but non-supplemented rations.

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## THE RELATION OF CHLORINE AND CATALASE CONTENT OF MILK TO ITS CURD TENSION\*

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The type of curd obtained from milk with rennet has been studied by many in attempts to relate the nature of the curd to one or more of the properties of the milk. The reaction of the enzyme and the milk will vary from sample to sample in its speed, that is, in the time required for the rennet to cause visible curdling under standard conditions of temperature and concentration of enzyme. The physical properties of the coagulum will also vary from sample to sample. Most observations have been limited to the product of individual cows and also to relatively short periods. A field project on the manufacture of cheese presented an opportunity to study curdling properties and milk composition of each of six herds daily throughout a lactation period and the milk of each animal in each herd several times in the period.

The animals of the six herds, totaling 157 milking cows, freshened in early spring and were dried off in early November. The resting time between lactation periods was thus longer than in herds in which production is maintained at a relatively constant level throughout the year.

Firmness of the curd produced under standard conditions of temperature and concentration of rennet, chlorine and catalase content of the sample, and also the reducing power of each sample toward methylene blue and toward resazurin were observed. The accumulated experience of many has shown that the mass of reproducing bacteria of the milk is the chief reducing constituent of milk toward methylene blue and that the mass of growing bacteria and the mass of body cells in milk are the reducing agents toward resazurin. The action of each of these two agents on resazurin can be separately determined in some degree when the reducing power of the milk on this reagent is compared with the action on methylene blue. The reduction of resazurin can be divided into two phases: first, a change from the original slate gray color of the milk-resazurin mixture to a pink, and second, a change of the latter to white. The extent of the progress in each phase can be observed. If the content of a milk in body cells (leucocytes) or bacteria is high, the time required for the first phase of the reduction of resazurin will be short. The answer as to which of the reducing agents is responsible for a color change noted with resazurin is supplied by comparing results of the two

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tests. If the bacteria are the chief cause of reduction in any sample, reduction times with the two dyes will be much the same. If, however, the methylene blue indicates presence of few bacteria, then the rapid reduction of resazurin to its pink form is due to body cells, the presence of which is indicated by the high catalase content, since the body cells are apparently the source of the catalase of milk. Its other constituents are devoid of this enzyme. The chlorine content of milk high in catalase will also be high since blood serum will also be entering the milk system if cells are passing from the blood system into the milk system. Thus chlorine, catalase and body cells will tend to vary together in the milk of an individual cow and these variations will cause variations in reducing power of the milk toward resazurin. High bacterial content will tend to obscure the latter relation.

The entrance of blood serum into the milk system tends to raise the pH of the milk and increase the sodium: calcium ratio, changes that alter the

TABLE 1  
*The season's history of the herds studied*

Herd	May 9				Sept. 30				Oct. 24			
	Cows		Milk		Cows		Milk		Cows		Milk	
	No.	%	lbs.	%	No.	%	lbs.	%	No.	%	lbs.	%
1	34	100	1120	100	24	70	576	60	20	60	364	32
2	33	100	902	100	31	94	446	49	31	91	324	36
3	27	100	791	100	27	100	569	72	25	92	387	49
4	21	100	450	100	17	81	235	52	14	66	242	53
5	24	100	576	100	21	88	497	86	21	88	314	54
6	18	100	606	100	17	94	360	57	15	83	258	42
Average	157	100	741	100	137	87	447	60	126	80	315	42

speed of reaction of milk with rennet and also the properties of the coagulum. It seems probable that the presence of an inflammatory process in the udder causes a decrease in the percentage of casein from that normal for the animal and changes its quality in such a manner that curdling with rennet is retarded and the firmness of the curd is decreased from that normal for the animal. It is also to be remembered that the curds formed in the milk of different cows by rennet will vary in physical properties due to the genetic pattern of the animal. In this case the four quarters of the udder should produce milk showing the same type of coagulum, while in the case of an inflammatory process the nature of the coagulum noted in the product of the affected quarter or quarters would differ from that of the normal quarter.

This study is one of the relation of mastitis to the quality of milk and to the quality of the cheese made therefrom. The discussion is based wholly on comparative values, so it does not seem essential to describe in detail the methods used. The property of the curd observed was firmness or tension.

The seasonal record of the herds is presented in table 1.

The curd tension of the entire quantity of milk produced in 24 hours was noted once or twice each week throughout the season. Readings were made 30 and 40 minutes after the rennet was added to the milk. The relative values were the same in either case. Only those noted after 30 minutes are given in table 2. The product of herd 4 is markedly different in curd tension from that of the other herds.

In the detailed study of each herd it seemed that the clearest picture of the relation of mastitis to curd tension and to other items of interest would be obtained by comparing animals that in May produced milk of a curd tension of 5 or below with animals that during the same period produced milk having a curd tension of 40 or above. One point of interest is the relation of curd tension to persistence in production. The data are presented in table 3. The significance of the data is only indicative on account of the limited number of animals in each herd.

TABLE 2  
*Average curd tension by months of herd milk*

Herd	1	2	3	4	5	6
May .....	13	17	18	22	12	14
June .....	9	9	6	13	3	4
July .....	10	12	5	14	4	8
August .....	4	6	5	11	2	4
September .....	5	7	4	16	2	5
October .....	12	14	12	20	10	9
Average .....	8.8	10.8	8.3	16.0	5.5	7.3

The entire comparison may be thus expressed. Twenty-five cows that produced milk of a curd tension of 5 or below in May gave in late September 53.1 per cent as much milk as in May. Twenty-four cows that in May produced milk with a curd tension of 40 or above gave in September 65.0 per cent as much milk as in May. The observations conform with what one would expect in showing that mastitis is a factor in persistence of production in any lactation period.

#### CURD TENSION IN RELATION TO CHLORINE AND CATALASE CONTENT

The relationship of curd tension to chlorine and catalase content of milk was also studied, using only the extreme cases, *i.e.*, those with a curd tension of 5 or below and those with a curd tension of 40 or above. The judgment as to the members of the intermediate group would be questionable, hence the use of the extremes rather than the entire number of cows in the six herds.

The catalase content of the milks was determined by collecting the total amount of oxygen set free from an excess of hydrogen peroxide by a quantity of the milk and expressing it in terms of percentage of the quantity of milk

TABLE 3  
*The relationship of curd tension to persistence in production*

	Curd tension	On Sept. 25 produced of the May 8 yield
Herd 1		<i>Per cent</i>
3 cows	5 or less	24.7
3 cows	40 or more	68.0
Herd 2		
9 cows	5 or less	42
3 cows	40 or more	50.0
Herd 3		
5 cows	5 or less	74
3 cows	40 or more	60
Herd 4		
0 cows	5 or less	...
6 cows	40 or more	65
Herd 5		
7 cows	5 or less	92
2 cows	40 or more	100
Herd 6		
1 cow	5 or less	33
7 cows	40 or more	48

used, thus if 10 cc. of milk set free 10 cc. of oxygen its catalase value would be 100.

The values given in table 4 are the average of all observations during the entire period of observation. The relation of high chlorine and catalase content to low curd tension is to be noted in each herd except herd 5. Since the record of this herd differs so markedly from that of the other five herds, the detailed data are presented in table 5. Herd 5 contained many cows producing milk of low curd tension with no evidence of udder trouble as shown by the chlorine and catalase content of the milk. It has long been known that some cows normally produce soft-curd milk, while in others the changed composition of the milk is due to a disturbance in the physiology of the

TABLE 4  
*The catalase and chlorine content of herd milk of low and  
high curd tension in May*

Herd	Curd tension			
	5 or less		40 or more	
	Chlorine	Catalase	Chlorine	Catalase
	%	%	%	%
1	0.169	145	0.117	34
2	0.170	97	0.125	38
3	0.146	52	0.128	15
4	.....	.....	0.117	26
5	0.114	18	0.130	10
6	0.184	59	0.121	22



udder. As far as the writers know, this is the first instance of a herd of apparently normal cows producing milk of low curd tension or soft-curd milk. The herds studied consist largely, if not wholly, of their own progeny; the genetic factor responsible for soft-curd milk may thus be brought to attention in a degree not probable in a herd maintained by purchase.

TABLE 5

*The catalase and chlorine content of the milk of individual cows of low and high curd tension in herd 5*

Cow	Curd tension	Chlorine	Catalase	Cow	Curd tension	Chlorine	Catalase
May 9, 1941							
23	0	0.114	17	1	63	0.135	10
				5	48	0.122	14
				12	42	0.133	6
May 26, 1941							
6	0	0.085	7	2	48	0.088	2
13	0	0.102	15				
14	0	0.091	4				
15	0	0.097	28				
18	0	0.091	4				
19	0	0.093	17				
22	0	0.101	5				
23	0	0.083	10				
25	0	0.092	6				
27	0	0.089	35				
July 21, 1941							
6	0	0.082	12	1	42	0.087	17
7	0	0.100	3	2	52	0.082	2
14	0	0.102	6	4	65	0.101	...
15	0	0.079	50	5	43	0.086	5
18	0	0.103	10	11	40	0.098	14
22	2	0.103	9				
23	0	0.099	7				
27	5	.....	18				
				17	44	0.103	9
				24	43	0.103	33
Aug. 7, 1941							
6	0	0.079	21	2	65	0.081	5
7	0	0.066	10	12	52	0.062	14
14	0	0.086	18	17	50	0.097	13
15	0	0.100	69	24	42	0.106	36
18	0	0.107	17				
22	0	0.087	39				
23	0	0.084	30				
27	4	0.090	47				

The resazurin tests were observed at the end of each hour for five hours, and a number given to each sample at each observation. A low number, 1, for example, indicates that little change in the color of the dye was noticed

TABLE 6

*The relationship of resazurin reduction after 1 hour incubation to curd tension, chlorine and catalase content of milk*

Herd	Curd tension	Cows	Chlorine	Catalase	Resazurin
1	5 or less	6	0.169	145	4.0
1	40 or more	10	0.117	34	2.0
2	5 or less	15	0.170	97	3.5
2	40 or more	3	0.125	38	1.0
3	5 or less	6	0.146	52	1.6
3	40 or more	3	0.128	15	1.0
4	5 or less	0	.....	.....	.....
4	40 or more	7	0.117	26	1.5
5	5 or less	1	0.114	17	1.0
5	40 or more	3	0.130	10	1.3
6	5 or less	2	0.184	59	4.0
6	40 or more	6	0.121	22	1.5

at that period of observation, while a high number indicated much greater reduction at the period of observation. Thus, 1 at the fourth hour means a milk very low in bacterial content and in cells, while 5 at the first hour means a milk high in bacteria or in body cells, or in both. The answer as to which is given by the methylene blue test. The milks in question were constantly so low in bacteria that a rapid reduction with resazurin may be used as an evidence of high content in body cells. It thus seems proper to use the averages of the resazurin reading of the two classes of cows—those producing milk of a curd tension of 5 or below and those showing a tension of 40 or above.

Table 6 presents the average reading of the resazurin test at the end of the first hour for the two groups of cows in each of the six herds on a particular day in May. The expected relation between high chlorine and catalase content and rapid reduction of resazurin is shown in each herd except 5, in which the more rapid reduction was noted in the high tension group of cows. The number of cows in each group of this herd was so small that the figures can be only suggestive. The smallness of the groups representing the extreme high and low values indicates that the herd consists largely of normal animals.

TABLE 7

*The relationship of catalase content of milk to the rapidity of reduction of resazurin*

Herd	Average catalase content	Average reading in resazurin test, 1 hr.
1	56	3.0
2	65	3.2
3	48	2.5
4	29	1.9
5	25	1.4
6	48	2.7

The relationship between the average catalase content of the entire amount of milk delivered at the factory to the average reading of the resazurin test at the end of the first hour for each herd is shown in table 7.

The relationship between catalase content and reducing power toward resazurin is clearly evident from the data, which also show that herd 5 is the only one of the six that can be considered as producing normal milk, since the milk of cows with healthy udders should not exceed 25 in its catalase value. Indeed, the value 20 is most frequently found in the literature on the subject, and in the experience of the writers some herds will consistently produce milk that will not exceed this value. The same is true of most first lactation cows.

The cause of the condition noted in herds 1, 2, 3 and 6 cannot be stated with certainty. From the few observations made as to the presence of streptococci in the milk of individual cows, one is inclined to consider them as the prime disturbing agent. The observations of the senior author indicate that herds in similar condition are to be found in all areas. It is hoped that the incidence of such herds is not as high as in the six studied, for they cannot serve as the foundation of a successful farm or of a successful cheese industry. Some program of herd management should be developed through the use of which such herds can be placed on a more normal base.

#### SUMMARY

The tension of the curd produced under standard conditions in the 24-hour product of each of six herds was noted frequently throughout a period of six months. The product of one herd (No. 4) had an average curd tension approximately twice as great as that of the other five herds and is believed to represent what one would expect to obtain from a group of cows with healthy udders. The low curd tension of four of the remaining five herds is believed to be due to the high incidence of chronic mastitis in these herds, since the product thereof was abnormally high in catalase and in chlorine. The low curd tension noted in the product of the remaining herd (No. 5) is believed due to the genetic pattern of the members of the herd since the chlorine and catalase content of its product was normal.

The record of the four herds indicates them to be agents of low value for the production of milk both from the standpoint of quantity and quality of milk.



## THE VISCOSITY OF EVAPORATED MILKS OF DIFFERENT SOLIDS CONCENTRATION

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The viscosity of evaporated milk is an important characteristic because, in the minds of many consumers, it indicates a quality of "richness." Of greater significance, however, is the fact that an increase in the viscosity of the evaporated milk retards the rate of fat-separation in storage, since the phenomenon of fat-globule-rise is largely a function of size of globules and viscosity of medium. Objectionable fat-separation may occur because of improper homogenization of the milk or because the body of the evaporated product is too "thin." This report is a discussion of a preliminary study of the viscosity of evaporated milks of different concentrations and of the effects of different manufacturing and storage conditions.

Some years ago preliminary observations were made in these laboratories on evaporated milks that contained approximately 26 per cent of milk solids. Forewarming the whole milks up to 100° C. (212° F.) was carried out in a steam-jacketed hotwell; forewarming at 100° C. to 120° C. (248° F.) was accomplished in an autoclave. The reciprocal relationship of heat stability and consistency was noted in many instances. For example, forewarming a milk at 70° C. (158° F.) for 10 minutes usually rendered its evaporated product less stable than an evaporated product from a milk heated to 95° C. (203° F.) for 10 minutes. However, as a rule the consistency of the former product at coagulation was greater. In several cases, milks heated to 105° C. (221° F.) for 10 minutes produced evaporated products of greater stability than those from other samples forewarmed at lower temperatures. The consistencies of the milks heated to the higher temperatures were less.

These results were similar to those obtained by Grindrod (1), who subjected milk to the "impact process" of sterilization and found that milk heated to 110° C. for one minute possessed greater heat stability than did milk forewarmed at the commercially used temperatures (90°-100° C.). The resulting evaporated milk was too fluid.

Hunziker (2) states that forewarming temperatures above the boiling point produce maximum stability but low viscosity while the reverse is true at lower forewarming temperatures. Recent work in these laboratories (5, 6) has shown that the heat stability and concentration of evaporated milks can be greatly increased by the use of high temperatures applied to the milk for a short time. By means of this method of treatment, samples with a wide range of concentrations and stabilities were made available for viscosity determinations.

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## EXPERIMENTAL

Whole milk from the Beltsville herd of the Bureau of Dairy Industry was standardized to a fat to solids-not-fat ratio of 1 to 2.29. Experimental samples were prepared, both with the usual forewarming and with the high-temperature forewarming procedure described elsewhere (5, 6). The milks forewarmed at 95° C. for 10 minutes were heated in a steam-jacketed hotwell. Those forewarmed by the high-temperature short-time method were heated in a Mallory heater (3).

After the milk was forewarmed it was concentrated in a stainless steel vacuum pan to 32–35 per cent solids, heated to 60° C., homogenized at a pressure of 2500 pounds per square inch, and cooled. Precautions were taken throughout the work to obtain uniform homogenization in all batches and thus to eliminate any variable which might result from a lack of uniformity in fat-globule size. Small quantities of the milk were diluted with water to the desired solids content. The heat stability, or time required to initiate coagulation, in these samples at 115° C. was then determined by heating them in small cans in a pilot sterilizer. The sterilizer reel revolved at the rate of 4 rpm. and during the treatment of all samples it was allowed to run continuously.

Viscosity determinations were made on 150-gram samples with a McMichael viscosimeter. The measurements were made at 20° C., unless otherwise stated. The wires were standardized against sugar solutions of known viscosity and all results were converted to centipoises. The cans of milk were stored in constant-temperature rooms, undisturbed, until viscosity determinations were to be made. Samples were adjusted to the temperature of measurement by immersing the cans in a water bath for several hours before opening them. Excessive fat-separation in occasional samples made it impossible to obtain accurate measurements on such milks, and consequently some inconsistencies in the data were caused by this change in fat-dispersion.

## RESULTS

The data plotted in figures 1 and 2 show the course of viscosity development in concentrated milks of different types during heating to and beyond the point of coagulation. The effect of aging milk and of the addition of lactic acid upon the body produced during sterilization is shown in figure 1. The curves in figure 2 show the viscosities of milks of different concentrations after various periods of heating at 115° C. The samples of figure 1 were forewarmed by usual commercial methods (95° C. for 10 minutes) while those of figure 2 (except the "95°" controls) were forewarmed at high temperatures and held for 25 seconds. These figures indicate how rapidly the viscosity of the evaporated milks increased just before and after coagulation. The viscosities at coagulation decreased as the heat stabilities of the milks increased.

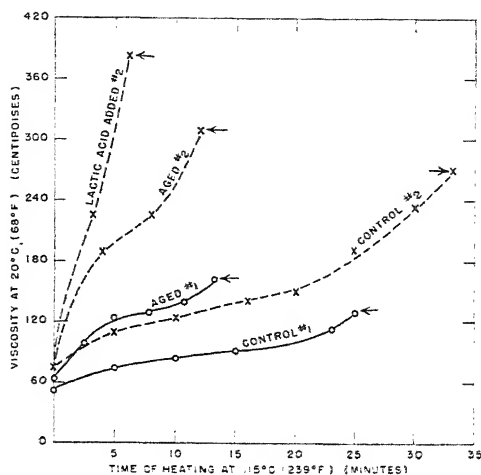


FIG. 1. The relationship between the heat stability of concentrated milk and the viscosity which is developed in it during heating at 115° C. (239° F.). The arrows indicate the time of the appearance of visible coagulation. All milks were forewarmed at 95° C. (203° F.) for 10 minutes before concentration to 26 per cent solids content.

The relationship between the viscosities of evaporated milks of different solids content and their heat stabilities is shown in figure 3. The body of the

TABLE 1

*The effect of differences in heat stability upon the viscosity of evaporated milks sterilized at 115° C. (239° F.) for 18 minutes and held in storage. The different stabilizing heat treatments used on the raw and concentrated milks are indicated. Samples bearing the same date were prepared from a common sample of raw milk.*

Date	Pre-sterilization heat treatment of milk				Total solids	Heat stability	Viscosity at 30° C. (86° F.) after storage at 30° C.				
	Raw milk		Concentrated milk				No storage	10 days	30 days	225 days	410 days
	Temp.	Time	Temp.	Time							
	°C.	min.	°C.	min.	%	min.	c.p.	c.p.	c.p.	c.p.	c.p.
12/2/41	95	10	.....	.....	26.0	44	26	12	13	13	.....
12/29/41	140	1	.....	.....	31.7	23	174	95	47	51	.....
2/13/42	95	10	.....	.....	26.0	43	30	19	16	24	.....
	120	2	.....	.....	34.9	25	460	222	156	360	.....
6/25/42	95	10	.....	.....	27.7	23	58	24	19	18	15
	95	10	120	0	27.7	34	25	16	15	14	15
	95	10	130	0	32.5	44	66	43	37	53	55
	95	10	140	0	37.2	35	165	132	121	213	191
7/9/42	95	10	120	4	35.0	22	207	165	170	197	290
7/13/42	95	10	.....	.....	27.6	29	45	18	16	15	17
	95	10	120	0	27.6	46	24	14	13	18	20
	120	1/4	.....	.....	27.6	36	34	14	13	16	49

milk was greatly influenced by the heat stability of the sample while one of the important factors affecting stability was concentration. The effect of heat stability upon the viscosity of some milks of different concentrations is shown also in table 1.

Results which show the extent of the viscosity changes in evaporated milk subjected to different manufacturing and storage conditions are presented in figures 4 and 5 and in table 1. The important changes during storage

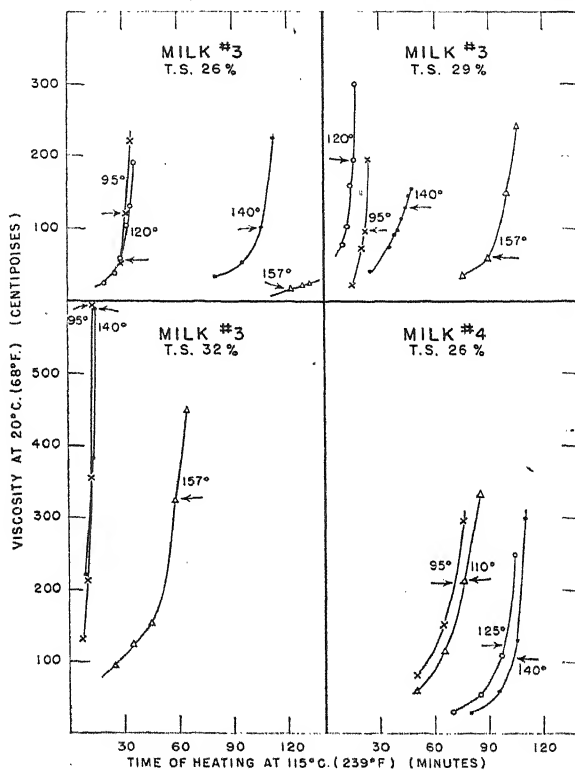


FIG. 2. The relationship between forewarming temperature, heat stability and the development of viscosity in two evaporated milks during sterilization. The solids content of the milk is indicated in each quadrant. The forewarming temperature is given for each curve; the forewarming time of the 95° C. samples was 10 minutes while for the other samples it was 25 seconds. The arrows indicate the time of the appearance of visible coagulation.

were: a thinning of all samples during early storage, then a period of only slight viscosity change, and finally a thickening in some of the milks.

The initial loss in body in all the milks tested was retarded by low storage temperatures. Samples of a representative 26 per cent solids milk held 55 days at 25° C. decreased in viscosity to the same extent as other samples held 8 days at 40° C. Samples held 55 days at 16° C. had the same viscosity as those held 5 days at 25° C.



Data on the effect of aging whole milk upon the viscosity of its 26 per cent evaporated milk were obtained. Fresh milk was held at 37° C. for 2 hours during which time the acidity increased from 0.18 to 0.21 per cent. Aging the fresh milk affected the viscosity of its evaporated product indirectly through its effect upon heat stability. Thinning of the milks during storage did not appear to be caused by the aging treatment.

The differences in the kind of body which may be expected in storage samples of evaporated milk of approximately 30 per cent solids content is

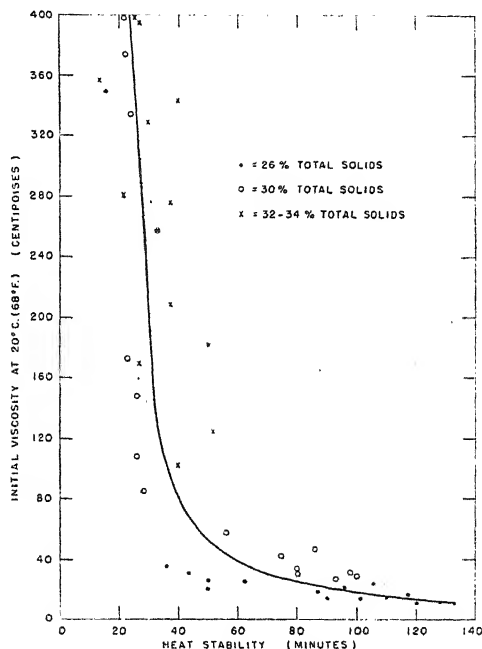


FIG. 3. Viscosities of evaporated milks of different heat stabilities measured after completion of the normal sterilizing process. Each milk was cooked for 18 minutes at 115° C. (239° F.), cooled to 20° C. and the viscosity determined within 3 hours after processing.

shown in figure 4. Milk A showed progressive thickening after completion of the initial thinning reaction, while milk B remained substantially unchanged in viscosity during the storage period.

Data on forewarming treatment, solids content, heat stability and viscosity of six batches of milk are given in table 1. The samples were stored and viscosity measurements were made at 30° C. The loss in the viscosity of these samples after 10 days' storage at 30° C. averaged 41 per cent of the original viscosity values. There was an additional average loss of 10 per cent of the original values during storage between the 10-day and 30-day periods. These losses are of the same order as those reported by Mojonnier and Troy (4).

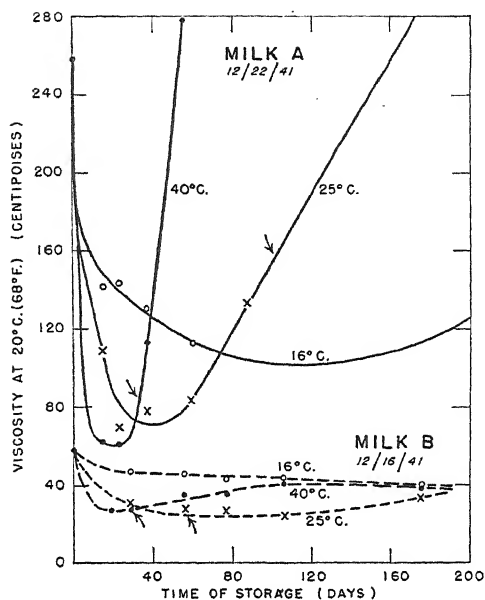


FIG. 4. The effect of time and temperature of storage upon the viscosity of two evaporated milks. The time of observance of heavy fat separation is indicated by arrows. Only moderate fat separation was noted in the 16° C. samples at the end of the storage period. Sample 12/16/41 was forewarmed at 140° C. (284° F.) for 30 seconds, contained 30.0 per cent solids and had a heat stability of 56 minutes. Sample 12/22/41 was forewarmed at 130° C. (266° F.) for 30 seconds, contained 31.7 per cent solids and had a heat stability of 33 minutes.

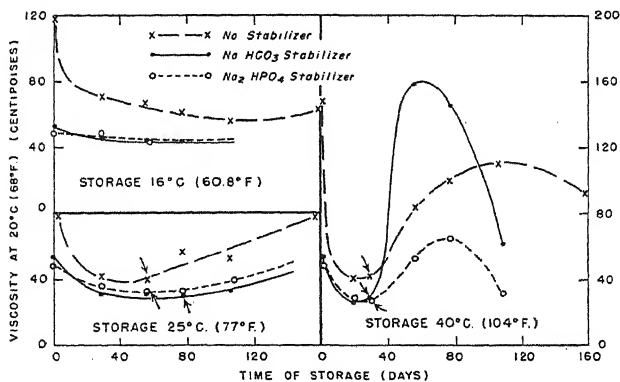


FIG. 5. The effect of time and temperature of storage upon the viscosity of samples of an evaporated milk sterilized with and without stabilizers. The time of observance of heavy fat separation is indicated by arrows. Only moderate fat separation was noted in the 16° C. samples at the end of the storage period. The milk was forewarmed at 95° C. for 10 minutes before concentration to 30 per cent solids content. The heat stabilities of the samples were: no stabilizer—26 min., bicarbonate stabilizer (4½ oz. per 1000 lbs. milk)—34 min., phosphate stabilizer (18 oz. per 1000 lbs. milk)—37 min.

The effect of large quantities of two stabilizing salts on the body changes of an evaporated milk during storage at three temperatures is shown in figure 5. This milk was representative of six samples upon which data of this nature were obtained. The high viscosity of the control sample can be attributed to its low stability.

Observations on fat-separation during storage were made on the evaporated milk samples in conjunction with the viscosity determinations. Figure 6 shows in a general way the relationships between temperature and time of storage. The curve for excessive fat-separation was based on visual observations; exact measurements were not made. If it is considered that cans of evaporated milk should be turned when they have stood half the time

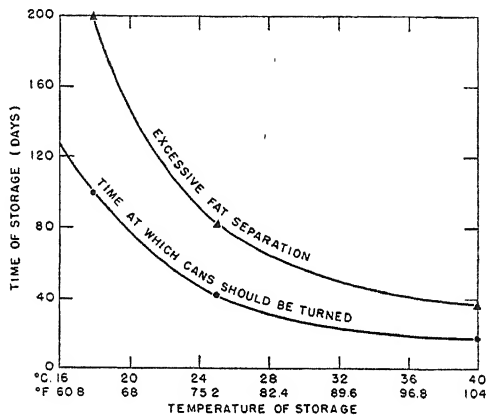


FIG. 6. The effect of temperature and time of storage upon fat separation in evaporated milks. The upper curve is based on average values from 20 milks of 26–32 per cent solids content held in undisturbed storage at different temperatures. The lower curve is an estimate of the time at which cases of evaporated milk in storage should be turned.

necessary to cause excessive fat-separation, the lower curve of figure 6 may be drawn. It would, therefore, seem reasonable to require the turning of cases of evaporated milk at some regular interval which lies below the lower curve of figure 6. For example, at 25° C. (77° F.) the milk should be turned every 6 weeks.

#### DISCUSSION

The viscosity of evaporated milk passes through well-defined changes arising from the manufacturing processes and the storage conditions to which it is subjected. There is first a thickening during the heat of sterilization, then a thinning during early storage and finally, after long periods of storage, the viscosity may again increase until a gel has formed.

The rate of thickening of evaporated milk during the sterilization process is variable, being the greatest shortly after heating is begun and immediately before coagulation appears. While the rapid thickening immediately pre-

ceding visible coagulation is, no doubt, a part of the coagulation process, all milks do not attain the same body before coagulation begins. The attainable body at coagulation seems to depend primarily upon the heat stability of the milk. Heat stability, in turn, is largely influenced by the concentration of milk solids, the conditions of forewarming, and the ionic equilibrium which exists in the serum.

The results of a number of experiments with milks differing in quality and forewarmed at different temperatures indicate that the relationship between the attainable viscosity at coagulation and the heat stability of the evaporated product is of a reciprocal nature.

Thickening in evaporated milks during sterilization does not proceed rapidly until about 10 minutes before coagulation. A processing period of about 20 minutes is generally favored in commercial practice. If a heavy, creamy body is to be developed, the heat stability of the milk should not exceed 30 to 40 minutes. When the cooking time to be used approaches the heat-stability time, extensive increases in viscosity may be encountered. Figures 1 and 2 show the thickening for which each minute of heating may be responsible when the milk is in the pre-coagulation phase. Milks with stabilities in excess of 50 minutes will be exceedingly thin after heating them at 115° C. for 20 minutes unless an increase in solids content is depended upon to build body.

The first change which occurs in the body of evaporated milk during storage is a rapid thinning, an effect which is accelerated at high storage temperatures. As thinning progresses a viscosity level is finally reached which may be termed the basic storage viscosity. In this region the viscosity ceases to decrease so rapidly; there is a distinct flattening in the viscosity curve (figs. 4 and 5, and table 1). As the milk loses body, another storage change, a separation of the fat, begins.

During storage, control of the body of evaporated milk should be first concerned with methods for delaying the initial thinning process and establishing a relatively high basic storage viscosity. Attainment of the basic storage viscosity may be effectively delayed by subjecting the milk to low storage temperatures. When evaporated milks were stored 60 days at 20° C. (68° F.) or lower, the loss in viscosity was less than the loss which occurred after 10 days at 40° C. (104° F.). Evaporated milks subjected to storage temperatures above 30° C. (86° F.) often reached their basic storage viscosity in 10 days.

Although the level of the basic storage viscosity was determined largely by heat stability, it was also influenced by the solids content of the milk. The 37 per cent solids milk of June 25, table 1, had a stability of 35 minutes and a viscosity after 30 days' storage of 121 centipoises. A portion of the same milk forewarmed at 120° C. (with no holding time) to give the same stability (34 minutes) at 27 per cent solids content had a viscosity of only 15 centipoises after 30 days' storage.

No manufacturing treatment, such as alteration in the type or conditions of forewarming, has yet been found which proved capable of preventing the initial rapid loss in the viscosity of evaporated milk during storage at high temperatures.

The last stage in body deterioration of evaporated milk consists of a thickening of the milk, which sometimes reaches the point of gelation. Storage thickening is rarely found in the commercial product, partly because much of the milk is consumed before thickening sets in and partly because some milks apparently never would reach this phase of development. Before thickening becomes objectionable an excessive fat-separation can generally be observed. The viscosity increase during storage usually starts too late to be very helpful in retarding fat-separation.

Certain samples, such as milk B, figure 4, did not thicken. The storage viscosity curves of many 26 per cent evaporated milks followed the course of milk B. High-solids milks thickened more readily than did 26 per cent milks but the greatest increase occurred in the solids range above 32 per cent.

The use of sodium bicarbonate as a heat stabilizer (fig. 5) accelerated storage thickening in several milks but not before a heavy layer of fat separated from the samples. Sodium bicarbonate is no longer used as a stabilizer in the manufacture of evaporated milk.

Evaporated milks which have been subjected to sterilization processes requiring only a minute or two to complete and consequently which have little cooked flavor and color are known to thicken quickly during storage. Evidently the drastic heat treatment of the commercial product retards storage thickening while the mild treatment given various experimental batches permits it.

Little is known of the real cause of thickening in evaporated milk during storage. It may be a slow continuation of the coagulation process accompanied by an orientation of the caseinate molecules which finally produces an irreversible gel structure. Molecular orientation is suspected as being involved because undisturbed storage appears to promote thickening and the early incipient gel structure may be easily reduced by shaking.

It is hoped that future investigations on the viscosity of evaporated milk will eventually indicate a means of controlling the thinning and thickening processes so that a good body in evaporated milk can be produced and permanently held during storage.

#### SUMMARY

1. The viscosities of evaporated milks of 26 to 36 per cent solids concentrations followed a well-defined pattern during processing and storage. Thickening occurred during the sterilization process; this was followed by a loss of body and a thinning early in the storage period. The low storage viscosity was maintained for various and unpredictable lengths of time, dur-

ing which fat-separation occurred. Late in the storage period a final thickening, which often proceeded to gelation, sometimes set in.

2. The rate of thickening of evaporated milk during sterilization at 115° C. (239° F.), although variable, becomes greatest in the 10-minute period immediately preceding coagulation. A heavy, creamy body could be developed during this pre-coagulation stage. In general, the lower the heat stability of a milk, the higher the viscosity it would develop during the 10-minute period preceding coagulation. Concentrated milks of high heat stability which reached the end of the sterilization period before entering the thickening phase did not develop, during processing, the high viscosity shown by milks of lower heat stability.

3. The body formed during the sterilization of milks of different concentrations became greater as the solids content of the milk was increased. The heaviest viscosities were produced in milks of high concentration and low heat stability.

4. Variations in milk quality and in forewarming procedures affected the viscosity of evaporated milk indirectly through their effect upon the heat stability of the milk.

5. The magnitude of the decrease in the viscosity of evaporated milk during early storage was dependent upon the storage temperature. At temperatures below 16° C. (60.8° F.) the loss of body was small. At temperatures above 30° C. (86° F.) evaporated milk sometimes lost 40 per cent of its original viscosity during the first 10 days of storage.

6. After the initial thinning in the viscosity of evaporated milk held in storage at constant temperature, a basic storage viscosity level was reached beyond which the rate of viscosity loss during prolonged storage was small.

7. Some evaporated milks, especially those receiving light heat treatments and those with a high concentration of solids, began to show increases in viscosity even to the point of gelation, late in the storage period.

8. The results indicate that the procedure for developing and maintaining a satisfactory body in evaporated milk which is used by many manufacturers conforms to the best practice that can be devised from our present state of knowledge. Assuming efficient homogenization and uniform handling of the canned product, important steps in body control are: (a) adjust the heat stability to a value of a few minutes greater but not more than twice the cooking time, bearing in mind that viscosity increases rapidly during the 10 minutes preceding coagulation; (b) store the finished milk at a temperature below 21° C. (70° F.); (c) turn the cases approximately every six weeks; (d) the viscosity may be increased by raising the milk solids content of the product.

#### ACKNOWLEDGMENT

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## THE BACTERIOLOGY OF BULL SEMEN. II. THE EFFECT OF BACTERIA UPON RAPID TESTS FOR SEMEN QUALITY

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In a recent report Beck and Salisbury (1) have proposed two rapid methods for estimating the quality of bull semen; namely, the rate of methylene blue reduction by semen in yolk-citrate diluent and the survival of spermatozoan motility during short-time-high-temperature incubation. The following work was undertaken to determine the effect of bacterial contamination upon the accuracy and validity of these quick tests, especially the specific effect on the methylene blue reduction rate. The effect upon the methylene blue test was studied by two methods: first, by calculation of the multiple correlation coefficients to make allowance for the reduction due to spermatozoa and other known factors (1) in semen samples; and, second, by determining the methylene blue reduction time when pure cultures of bacteria were inoculated into yolk-citrate diluent, with and without seminal plasma. The effect of the short-time-high-temperature incubation test upon the bacterial content of semen has also been determined.

A preliminary estimate of the effect of bacteria upon the methylene blue reduction test may be obtained by an examination of the data available for other biological fluids, as for example milk. Thornton and Hastings (7) using 1:200,000 methylene blue at 37° C. found the bacterial count at the time of complete reduction to range from 3.5 to 45 million bacteria per ml. No sample with an initial count of fewer than 20 million bacteria per ml. gave a reduction time of less than one-half hour. In this and similar work the factors shown to affect the reduction time are the type of bacteria, their physiological condition, the temperature, the nature of the substrate present, and the concentration of methylene blue to be reduced. That storage may also be a factor was shown by Fayer (3) who stored milk samples at 4° C. for 18-72 hours and found a decrease in the rate of methylene blue reduction without a change in bacterial count. Bacterial counts of the magnitude usually found in semen samples would not on the basis of the findings of Thornton and Hastings (7) be expected to affect the proposed methylene blue reduction test.

### EXPERIMENTAL

#### *Methods*

The semen samples used in these experiments were obtained under routine conditions either from bulls in the dairy herd at Cornell University

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or from bulls owned by the New York Artificial Breeders' Cooperative in Syracuse, New York. All semen samples were collected with the artificial vagina. The semen was handled during examination, dilution and preparation for storage after the methods described by Willett and Salisbury (8). The methylene blue reduction tests were carried out as previously described (1). Briefly the test involves an observation of the time required for the complete reduction of 1:40,000 methylene blue by bull semen diluted at a constant rate with yolk-citrate diluent. With the exception of the experiments to determine the effect of various temperatures on the reduction time and upon bacterial numbers, the tests were run at 45° C. Bacterial counts were made on 2 per cent blood agar plates incubated 3 days at 37° C.

*The effect of bacteria present in semen on the reduction test*

For determination of the effect of bacteria, freshly diluted semen samples were selected from the bulls found in earlier studies (4) to yield semen containing the highest bacterial counts. Semen from these bulls was also of poorer than average quality in other respects; *i.e.*, long methylene blue reduction times had been noted. These samples were selected as those most likely to give erroneous estimates of semen quality if bacteria have a significant effect upon the methylene blue reduction test.

A factorial experiment was designed to simultaneously study the effect of the number of bacteria on the methylene blue reduction time at several temperatures and the effect of a 45-minute incubation period at these temperatures on the bacterial count. Samples of the same ejaculates were stored for 2, 4, 6, 8, and 10 days, following which bacterial counts and methylene blue reduction tests were run. Aliquots of the same samples were incubated and stored with and without methylene blue. In this way the effect of high-temperature incubation, apart from methylene blue, upon the bacterial count was determined. The methylene blue was added to one series of stored samples with the thought that if the reduction time was not influenced by bacterial growth, or other factors, the relative metabolic activity of the semen could be determined at any time during storage by simply warming a sample to the proper temperature and observing the reduction time.

For each ejaculate the spermatozoan concentration was determined at the time of collection and the motility was observed immediately before each methylene blue reduction test was performed. Thus, it was possible to determine the multiple effects of storage interval, spermatozoa count, motility, and bacterial count on the time required to reduce methylene blue. Ten ejaculates were used and 21 individual plate counts, motility estimates and methylene blue reduction tests were performed on each diluted ejaculate. It was later found necessary in the statistical analysis of the storage experiment to remove the data for two ejaculates as the semen was of such poor quality that it did not reduce the methylene blue in the time allotted for the

incubation period. The removal of these data had no influence on the conclusions drawn from these experiments.

For fresh samples the correlation coefficients between methylene reduction time for each of the following was: spermatozoan concentration,  $-0.7780$ ; spermatozoan motility,  $0.3431$ ; and bacterial count,  $-0.1427$ . The coefficient for spermatozoan concentration is the only significant observation ( $n = 10$ ). Though these fresh samples contained a wide range of bacterial counts the number present have but a minor influence on the methylene blue reduction time. The stored samples showed even wider variation in bacterial count. With these samples the calculated correlation coefficients for each of the following was: spermatozoan concentration,  $-0.6126$ ; spermatozoan motility,  $-0.3110$ ; and bacterial count,  $-0.1022$  ( $n = 48$ ). The first two coefficients are statistically significant, the last is not. Even with the larger number of observations the data indicate that the part played by bacteria in the reduction time is not large. All samples contained predominantly diphtheroid organisms, except one ejaculate in which the majority of the organisms were *Pseudomonas aeruginosa* (*pyocyaneus*).

The mean data for the fresh and stored samples are shown in tables 1 and 2. The methylene blue reduction times for fresh semen are too short for bacterial multiplication to be an important factor, when the tests are run at temperatures above  $45^{\circ}\text{C}$ . as recommended. In fact, the experiments show (table 1) a decrease in bacterial count at temperatures of  $45^{\circ}\text{C}$ . and above. The decrease is especially marked in the presence of methylene blue.

The stored samples (table 2) showed, on the average, a slight decrease in bacterial count over the first 4 days followed by an increase. Over this period, the methylene blue reduction time increased slightly and then decreased. The correlated changes are, however, too slight to be of significance.

#### *The effect of methylene blue on the numbers of bacteria*

From table 1 a comparison of the numbers of bacteria found in semen samples after 45 minutes incubation with and without methylene blue may be obtained. The samples used had stood at the laboratory temperature for about 30 minutes before incubation, which time was found to be the average required to set up the experiments. In table 2 may be seen a similar comparison of bacterial counts made on the same ejaculates after storage at  $5^{\circ}\text{C}$ . The methylene blue had been added to one series of samples before storage, and to the other just prior to the 45-minute incubation period. As limited reduction had proceeded in the samples to which methylene blue was added before storage, the reduction times were shorter than for the samples to which the reagent was added just before incubation.

It will be noted that incubation of the fresh semen in the presence of methylene blue reduced the bacterial count in all cases. In the absence of methylene blue, the count decreased only at the two higher temperatures. An analysis of variance of the data showed these differences to be highly

TABLE 1  
The effect of incubation temperatures on the bacterial count of semen samples

No. samples	Spermatozoa	Motility fresh	Bacterial count	Mean methylene blue reduction time during and mean bacterial counts after 45 minutes' incubation at											
				37.5°		40.0°		42.5°		45.0°		47.5°			
				Time	Count	Time	Count	Time	Count	Time	Count	Time	Count		
8	<i>thousands per mm.<sup>3</sup></i> 1109	%	<i>thousands per ml.</i> 640	<i>min.</i> 31.5	<i>thousands per ml.</i> 553	<i>min.</i> 29.6	<i>thousands per ml.</i> 624	<i>min.</i> 12.1	<i>thousands per ml.</i> 561	<i>min.</i> 9.7	<i>thousands per ml.</i> 463	<i>min.</i> 9.9	<i>thousands per ml.</i> 316		
				Mean bacterial counts after 45 minutes' incubation without methylene blue present											
				.....	879	.....	897	.....	884	.....	590	.....	503		

TABLE 2  
Mean reduction time and bacterial count after storage at 5° C.

Storage period	Spermatozoa motility	Methylene blue reduction time	Bacterial count after 45 minutes at 45° C.	
			Without methylene blue	Methylene blue added before storage
days	%	min.	thousands per ml.	thousands per ml.
0	66.2	9.7	640	640
2	47.5	14.7	420	363
4	37.5	12.9	307	237
6	31.2	11.1	1,141	567
8	30.0	12.1	3,171	1,054
10	25.0	13.2	2,888	2,979

significant. These facts support the use of 46.5° C., previously suggested by Beck and Salisbury (1), for the short-time-high-temperature incubation and methylene blue reduction tests. These workers have shown a very high correlation between the decrease in motility at 46.5° C. in 1 hour and the decrease in motility during 10 days' storage at 5° C. The bacterial count for samples stored with methylene blue was slightly lower in all cases than samples stored without methylene blue, but the difference was so small as to suggest that this was only a chance deviation. In the previous report (1) it was shown that the amount of methylene blue used did not have a detri-

TABLE 3

*Methylene blue reduction test on pure cultures of bacteria in yolk-citrate diluent*

Organism added	Days stored at 5° C.					
	0		5		10	
	Bac- terial count	Reduc- tion time	Bac- terial count	Reduc- tion time	Bac- terial count	Reduc- tion time
	<i>millions per ml.</i>	<i>min.</i>	<i>millions per ml.</i>	<i>min.</i>	<i>millions per ml.</i>	<i>min.</i>
<i>Escherichia coli</i> .....	130.0	.....	0.6	.....	11.0	100.0
	1,500.0	31	15.0	40	13.0	> 120.0
<i>Aerobacter aerogenes</i> .....	900.0	> 120	48.0	.....	320.0	7.5
			750.0	11	1,000.0	4.5
<i>Pseudomonas aeruginosa</i>	800.0	> 120	750.0	.....	36.0	10.0
			9.0	.....	120.0	6.0
<i>Staphylococcus aureus</i> ...	1,200.0	> 120	0.18	.....	9.0	14.0
			15.0	.....	6.0	.....
Diphtheroid strain 1 .....	2.0	.....	0.13	.....	0.3	.....
2 .....	7.0	.....	21.0	.....	4.0	.....
	<i>thou- sands per ml.</i>	<i>min.</i>	<i>thou- sands per ml.</i>	<i>min.</i>	<i>thou- sands per ml.</i>	<i>min.</i>
None (control) .....	0.03	.....	0.3	.....	4.0	.....

mental effect on the maintenance of spermatozoan motility in stored samples. From these observations it has been suggested that this dye could be used to identify the semen of one breed of bulls from that of another and thus avoid field errors which sometimes arise in artificial insemination.

*The effect of specific types of bacteria on methylene blue reduction  
in yolk-citrate diluent*

To test directly the effect of bacteria, yolk-citrate diluent and yolk-citrate diluent plus seminal plasma were inoculated with pure cultures in numbers higher than those usually found in semen and the methylene blue reduction test performed at 45° C. These tests were, as previously mentioned, performed within 30 minutes of inoculation. Other inoculated samples were stored for 5 and 10 days before reduction tests were performed. Twenty-

hour cultures of organisms isolated from semen samples were used as inoculum. These organisms were encountered in large numbers in various samples of semen during the course of the study. The data in tables 3 and 4 indicate that in fresh samples, none of the organisms would interfere with the test even when present in numbers as high as 1 billion per ml. The shortest reduction time (30 minutes) which was obtained with 1.5 billion *E. coli* was still beyond the reduction time for all but the poorest semen samples.

The results with samples stored for several days were not as satisfactory. If bacteria, which grow at the storage temperature, are present the reduction

TABLE 4  
*Methylene blue reduction test on pure cultures of bacteria in yolk-citrate plus seminal plasma*

Organism added	Days stored at 5° C.					
	0		5		10	
	Bacterial count	Reduction time	Bacterial count	Reduction time	Bacterial count	Reduction time
	<i>millions per ml.</i>	<i>min.</i>	<i>millions per ml.</i>	<i>min.</i>	<i>millions per ml.</i>	<i>min.</i>
<i>Escherichia coli</i> .....	48.0	60	54.0	48	75.0	9.0
	6.0	.....	7.0	.....	.....	.....
<i>Aerobacter aerogenes</i> .....	17.0	120	900.0	12	2,000.0	6.0
<i>Pseudomonas aeruginosa</i> ...	60.0	120	66.0	120	60.0	6.5
<i>Staphylococcus aureus</i> .....	8.0	120	30.0	120	20.0	16.0
Diphtheroid strain 1 .....	3.0	.....	6.0	.....	4.0	15.0
2 .....	0.02	.....	0.03	.....	0.05	40.0
	<i>thousands per ml.</i>	<i>min.</i>	<i>thousands per ml.</i>	<i>min.</i>	<i>thousands per ml.</i>	<i>min.</i>
None (control) .....	0.015	.....	0.009	.....	.....	.....

time may be very short. Cultures of *Aerobacter aerogenes* and *Pseudomonas aeruginosa* not only grow at 5° C. but the citrate present in the diluent is an excellent substrate for these organisms. When 750 million or more *Aerobacter aerogenes* organisms per ml. are present (tables 3 and 4), they would interfere markedly with the validity of the methylene reduction test after 5 days' storage at 5° C. After 10 days' storage counts of a hundred million of either of these two species of bacteria reduced the methylene blue fast enough to interfere with the test. When the seminal plasma was added to the yolk-citrate short reduction times were observed with each organism after a storage period of 10 days. The effects of the bacterial types contributed by the seminal plasma were not determined. However, as one may observe from the tables the number of bacteria in the plasma was very low. The range of bacterial numbers, in fresh diluted semen, the mean of which is shown in table 1, was from 97 thousand to 4.5 million.

After 10 days' storage the average count had increased to about 3 million bacteria per ml. with one count as high as 60 million. In only three diluted semen samples were the numbers as high after 10 days' storage as was found in the inoculated material. In three ejaculates the count increased as much as 20-fold, while in three others the total count decreased during storage. Greater increases in count were reported in a recent publication of the United States Department of Agriculture (5). With higher initial counts these workers report a 10-fold increase in bacterial count during 7 days and a 65-fold increase during 10 days' storage at 4° C.

A study of the data of this experiment reveals that only part of the short reduction times observed for stored yolk-citrate may be attributed to bacterial numbers. For diluent, or diluent plus seminal plasma, stored 10 days, short reduction times often were found where the numbers of bacteria present was no higher than those which in fresh yolk-citrate required more than 2 hours to reduce the dye. Apparently some factor other than numbers of bacteria present is partially responsible for the short reduction times observed for stored, inoculated material.

In this connection it should be pointed out that the negative correlation between spermatozoa count and methylene blue reduction time continued at the high level previously reported (1) regardless of the length of storage interval. Such definite trends were not noted for the relation between motility of the spermatozoa and methylene blue reduction time as the storage interval advanced. For the present, the methylene blue test for quality of semen should be used only with fresh semen and fresh diluent for the most satisfactory results.

#### DISCUSSION

From the results of these investigations it is concluded that if bull semen is collected and handled with proper sanitary precautions, and if the diluent is prepared following a definite aseptic routine (4) the number of bacteria in fresh semen and diluent will not interfere with the estimates of semen quality obtained with the methylene blue reduction test. In addition, the temperature recommended for conducting the test, 46.5° C., is high enough that bacteria are unable to grow. In fact, such a temperature has a lethal effect on the bacteria, especially in the presence of methylene blue.

Of the bacteria most likely to interfere with the test, the coli-aerogenes organisms can be largely excluded by sanitary care in preparation of equipment and during collection and handling of samples. The exclusion or control of the number of pseudomonas organisms is more difficult. These bacteria are sometimes found in large numbers and as the predominating type in the semen of some bulls. Ordinary methods of control do not influence the numbers of these bacteria for their source appears to be deep in the reproductive tract. Bulls harboring *Pseudomonas aeruginosa* in their reproductive tracts are likely to have poor breeding records (2, 4) and are poor risks for artificial insemination.

For stored samples of diluted semen the methylene blue reduction test should be used with caution. Certain unknown factors, in addition to the apparently minor effects of the number and kinds of bacteria, influence the test with semen samples stored more than 2 days so that reliable conclusions may not be drawn. For estimation of the quality of samples stored for 4 or more days observation of the duration of motility at 46.5° C. should give reliable estimates of potential livability of spermatozoa during continued storage at 5° C. Further work needs to be done before the factors influencing the reduction test in samples of diluted semen stored more than 2 to 4 days can be established.

#### SUMMARY

1. When sanitary precautions are observed, the number of bacteria found in freshly drawn semen or freshly prepared yolk-citrate diluent is not sufficient to interfere with the methylene blue reduction test for semen quality.

2. The short-time-high-temperature incubation test for semen quality may kill up to 50 per cent of the bacteria present when the test is run at 45° C. or above in the presence of methylene blue. A temperature of 46.5° C. is recommended.

3. The methylene blue reduction test is not recommended as a criterion of the quality of semen stored more than 2 days.

4. The short-time-high-temperature incubation test is recommended as a criterion of continued livability of the spermatozoa in stored semen samples.

5. Though the bacterial population of stored samples can be controlled by proper precautions, factors other than the number of bacteria, and the activity and concentration of the spermatozoa are involved in the methylene blue reduction rate of stored samples.

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## PEPPERGRASS SILAGE

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Recently attention has been directed toward the use of weed crops for forage purposes. At the Virginia Station alfalfa and brome grass were established to serve as supplementary pasture crops to be available in July and August when bluegrass is in low production. The alfalfa had been pastured off by May 20th and there was a heavy stand of peppergrass (*Lepidium virginicum*) remaining unpastured. There was entirely too much of this weed to permit it to go to seed. If clipped and allowed to remain on the ground the peppergrass would smother out the alfalfa. Mowing, raking and hauling away the peppergrass would be costly if no use were made of it.

Ten acres of peppergrass, yielding about 28 tons, were cut and ensiled with 50 pounds of molasses per ton as a preservative after due consideration of the possibility that the silage might be strong with peppergrass flavor.<sup>1</sup> Five acres were cut from the alfalfa field and some alfalfa was picked up by the mowing machine. The other five acres were cut from the brome grass field and some brome grass was clipped. It was estimated that the ensiled material was 80 per cent peppergrass. The seeds were immature but had the field been mowed and the peppergrass left on the ground the seeds would probably have matured sufficiently to germinate. When the material was cut into the silo there was sufficient volatile material in the air to irritate the eyes. The ensiled material kept well. After the silage had undergone fermentation there was no trace of the peppergrass flavor or odor remaining. Cows ate up to 50 pounds per day of the silage and appeared to relish it better than silage made from bluegrass with corn meal as a preservative. Samples of peppergrass in as nearly as possible the same stage of maturity as that which was ensiled were submitted to the Agricultural Chemistry Department for analysis. A sample of peppergrass silage containing about 20 per cent alfalfa was taken from the silo and submitted also. The analyses of these materials are shown in table 1 with the analysis of corn silage for comparison. Table 2 shows that the quality of peppergrass silage was satisfactory as there was no decline in production due to peppergrass silage. Cow number 6 was nearly dry and therefore any change in the feed would not have prevented a decline in the milk production of the cow.

The milk had no taste of peppergrass or indole. When the milk was

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TABLE 1

*Analyses of peppergrass and peppergrass silage compared with corn silage*

	Corn silage* (dent, well matured, well eared)	Peppergrass	Peppergrass silage
	%	%	%
Dry basis			
Protein .....	8.56	12.86	16.19
Fat .....	3.08	5.54	4.40
Crude fiber .....	21.92	34.17	23.63
Ash .....	5.48	9.32	.....
Nitrogen-free extract .....	60.95	38.11	40.62
Total dry matter .....	29.20	36.47	28.75
Phosphorus .....	0.21	0.23	0.42
Calcium .....	2.74	1.34	1.21
Potassium .....	0.96	3.00	2.41
Magnesium .....	.....	0.40	.....
Silica .....	.....	1.28	.....

The peppergrass was analyzed by Mr. H. H. Hill and the peppergrass silage by James F. Eheart, both of the Agricultural Chemistry Department. The peppergrass contained approximately 20 per cent alfalfa.

\* The values on the dry basis are computed from data of Morrison's Feeds and Feeding, 20th Edition.

TABLE 2

*The milk production of cows fed bluegrass silage and peppergrass silage*

Cow No.	Bluegrass silage		Peppergrass silage	
	Amounts of milk produced			
	May 2-16	May 17-31	June 1-15	June 16-30
	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
1	393.0	350.0	313.5	332.5
5	476.5	429.0	431.5	455.0
6	274.0	183.0	145.5	108.0
7	400.0	346.5	321.0	336.5
Average daily milk flow for 4 cows .....	27.6	23.4	21.6	22.0

The rate of decline of milk flow was reduced when bluegrass silage was replaced with peppergrass silage.

TABLE 3

*The quality of butter made from milk produced by cows fed peppergrass silage*

Lot No.	Age of cream in days	Condition of cream at churning	Butter
1	7	High acidity	Salted, old cream flavor, no peppergrass flavor
2	7	Low acidity	Unsalted, superior flavor, no peppergrass flavor
3	1	Sweet	Unsalted, excellent flavor, 92 score

separated the créam had a normal flavor. Butter was made from the cream without using a starter with the results shown in table 3.

Three judges scored the butter samples and no off-flavors attributable to the peppergrass were detected.

Evidently the substances producing the peppery flavor of the green material were oxidized in the fermentation process. The growing of peppergrass for silage is not advocated; however, crops that contain peppergrass may be ensiled without fear that dairy products will have an off-flavor as a result of their use.

One hundred peppergrass seeds were taken from the silage two weeks after ensiling for a germination test. There was no germination. Furthermore there was no germination of seed taken from the silo in early September, after the elapse of a normal rest period for these seeds.

#### SUMMARY

Ensiling peppergrass (*Lepidium virginicum*) with molasses as a preservative produced a palatable silage which after fermentation had no peppergrass flavor. Neither cream nor butter produced from the milk of cows fed this silage showed any off-flavor. Peppergrass seeds did not germinate two weeks after ensiling. Crops containing peppergrass may be ensiled without fear that off-flavor will result in the milk or milk products.

#### ACKNOWLEDGMENT

The assistance of Dr. C. C. Flora in producing the butter and judging the butter samples is gratefully acknowledged.



# A NEW QUANTITATIVE METHOD FOR ESTIMATION OF TOTAL COMBINED LENGTH OF MOLD FRAGMENTS IN BUTTER<sup>1</sup>

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The method described in this report resulted from studies on retention of mold fragments (mycelia)<sup>2</sup> by butter, buttermilk and wash water during manufacture of butter.

During these and previous studies (3) it became apparent that a more quantitative method for estimating content of mold hyphae or filaments in butter and other products would have to be developed before information could be obtained on mold retention by the various materials studied. Since the "mold mycelia value" of butter by the official method now in use is influenced primarily by the total mass of mold growth present, the logical quantitative method would be one that would measure the total length of all combined mold fragments in a measured sample. The difficulty of extracting the mold fragments renders a macroscopic approach impossible. The possibility of analyzing for some chemical constituent contained by the mold hyphae and foreign to butter appears remote at the present time. About the only possibility remaining is a microscopic method. The Wildman method (4) in use at present provides a comparative rather than quantitative value because it does not measure the total length of the combined filaments in a sample. A method was therefore developed in which the total combined length of the mold hyphae visible with low power (100×) magnification of the microscope was determined and expressed in millimeters per gram or mgm. Because the method has proven so convenient for certain studies on mold content of butter and other materials, it is being published with the hope that it may prove useful to other investigators in this field.

## DESCRIPTION OF METHOD

1. Thoroughly mix 1 g. butter with 9 ml. hot (70–75° C.) gum solution. (It is desirable to scrape off about one-eighth inch of the surface butter and

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<sup>2</sup> The term "mold mycelia" now commonly used in referring to the amount of dead mold filament in butter should perhaps, technically speaking, be changed to "mold hyphae" or "fragments of mold hyphae." A mold hypha (plural—hyphae) is a single thread or filament of mold. A mold mycelium (plural—mycelia) consists of a mass or network of interlacing filaments or hyphae. Therefore it is the filaments or hyphae or hyphae fragments that are counted in the Wildman method. Filaments, hyphae and mold fragments are used synonymously in this paper.

then obtain the one gram sample by scraping it from an area of a few square inches.)

2. Transfer 0.1 ml. to one side of a  $1 \times 3$  inch ( $26 \times 77$  mm.) slide.

3. Spread over exactly one-half of the slide. (One means of evenly distributing the preparation is to spread the material with a small, solid glass rod, first lengthwise and then crosswise. The preparation seems to distribute itself more evenly if, after spreading on the slide, it is placed for a few minutes on a level surface at room temperature before setting on a warm surface to dry.)

4. Dry on a warm surface.

5. Stain with Newman's (1) or a Modified Newman's stain. (The same precautions regarding drying of stains before washing to remove excess dye, should be observed as in the staining of milk films with Newman's stain. It is possible to wash the slides too long in removing the stain. A few trials will indicate the amount required. Nevertheless some mold hyphae fragments will not stain intensely and a knowledge of the microscopic appearance of mold hyphae is helpful in identifying them.)

6. Calibrate factor for low power objective of microscope. Include dilution of sample and area of slide.

7. Examine 25 or preferably 50 fields on each slide. If the sample contains much mold, 25 fields may be sufficient. Estimate, by means of a ruled ocular micrometer disc, the total length of mold filament for each field. (Appearance of the preparation under the microscope is often improved by covering it with a thin film of immersion oil. This is particularly true where the method has been applied to substances containing considerable protein, such as cream or buttermilk.)

8. Calculate average length of filament per field.

9. Multiply average length of filament per field by the factor.

10. Express results as millimeters per gram or preferably millimeters per mgm.

#### SAMPLE CALCULATION

A. Diameter of field = 1.66 mm.

B. Area of field =  $\pi r^2 = 2.164$  sq. mm.

C. Area of one-half slide = 1001 sq. mm.

Number of fields in one-half slide = 463.

D. Butter diluted 1 : 10; 0.1 ml. placed on slide.

Total dilution = 100 times.

E. Factor =  $C \times D = 46,300$ .

F. Average total mm. mold filament per field of sample preparation = 0.346.

G. Mm. mold per gm. =  $E \times F = 16,020$ .

H. Mm. mold per mgm. =  $\frac{E \times F}{1000} = 16.02$ .

Some comments might be made on certain steps in the procedure. The diluting medium was a 0.75 per cent carob bean gum solution similar to that used in the Wildman method (4). Other gum solutions that provide sufficient viscosity and a reasonably clear solution should be satisfactory. Among the preparations also used in these studies were the common adhesives or fixatives used to fix tissue sections to slides. No difficulty was experienced with cracking, peeling or slipping-off of films when carob bean gum solution was used and the above staining procedure followed and slides rinsed gently in a container of water.

Numerous trials indicated that 1 g. of butter in 9 ml. gum solution and 0.1 ml. of this on a slide provided about the optimum dilution. A greater amount of butter increased the difficulty of fat removal and too small a sample provided too little mold for counting.

A number of common staining techniques were tried, including the Gram stain, negative staining, and staining with methylene blue after defatting in xylol. None of these showed any advantage over the simple Newman method used for milk smears. Substitution of half of the methylene blue in the Newman stain with basic fuchsin resulted in a preparation that stained the mold more intensely on some of the slides. A better staining technique for this purpose can possibly be developed.

For a sample of high mold content 25 fields per slide should be sufficient to count; however, 50 fields is preferable for a sample with moderate or low mold content. On the basis of present official standards for butter, 20 mm. per mgm. would be considered a high count. Duplicate slides should be prepared and results averaged.

#### RESULTS

A number of trials were carried out to determine the reliability of the new method. These included checks between various slides from the same sample, different samples from the same print of butter and counts on the same prints by two technicians using different microscopes with separate preparation of samples and slides.

TABLE 1

*Results on different samples from the same prints of butter*

Mm. mold per mgm. butter					
Sample A			Sample B		
Slide 1	Slide 2	Ave.	Slide 1	Slide 2	Ave.
12.87	11.20	12.04	10.19	11.85	11.02
3.80	3.43	3.62	3.80	3.52	3.66
8.15	9.72	8.94	12.13	8.89	10.51
2.87	3.89	3.38	3.33	2.96	3.15
20.46	19.54	20.00	19.08	18.06	18.57
25.74	23.15	24.45	25.56	25.09	25.33

TABLE 2

*Counts with new method by two operators using different microscopes*

Sample No.	Total combined length of mold fragments	
	A	B
	<i>mm. per mgm.</i>	<i>mm. per mgm.</i>
320	16.56	16.56
321	48.85	48.76
322	21.25	25.76
323	7.82	8.10
324	16.74	20.24
325	14.17	12.70
326	21.16	22.45
327	11.87	10.76

The values shown in table 1 are from a trial to determine the consistency of results between duplicate samples. As the results indicate, with one exception, the variation between the four slides on the two samples is relatively small for a microbiological analysis. The mold content of different portions of a print of butter appeared fairly constant. Whether different portions of a commercial churning would check as well with this method has not been determined. The results of Claydon (2) indicate that some significant variations may occur due to chance variation of mold filaments in different portions of a churning.

Two different operators employing different microscopes and separate preparation of samples can obtain comparable results with the new method (table 2). The two technicians making these counts had received only a few hours training in appearance and detection of mold hyphae in butter. Again the variations occurring are no greater than would be expected in a microbiological analysis.

Comparisons between values with the Wildman and new methods are too few to enable definite conclusions, but those presented in tables 3 and 4 at least indicate in a general way what might be expected. There appears to be a rough correlation between the results until the mm. per mgm. reach

TABLE 3

*Comparative values with new method and Wildman method on one series of commercial butter samples*

Sample No.	New method	Wildman method
	<i>mm. per mgm.</i>	<i>per cent positive fields</i>
66	0.88	2
296	3.26	12
294	3.64	16
295	9.72	46
293	11.53	44
297	19.29	80
298	24.89	96
299	49.54	92
300	75.01	96



about 25, after which the Wildman method shows little increase. It should be emphasized that further comparisons in large number must be made before any definite correlation between the two methods can be worked out. However, it appears from these preliminary results that when the mold content reaches about 15 mm. per mgm., a sample runs a good chance of having a Wildman count of 60, and when the mold content reaches 20 mm. per mgm., a sample will usually be definitely illegal. At about 25 mm. per mgm. the Wildman count will vary from 75-100 per cent positive fields and remain at about this figure as the mm. per mgm. increase. Commercial butters with more than 40 mm. per mgm. are not uncommon.

Two samples with the same total length of mold filament per mgm. may exhibit considerably different Wildman values. This is demonstrated best by two samples one of which contained 12.84 mm. per mgm. total mold

TABLE 4

*Comparative values obtained on commercial butter samples with new method and Wildman method*

Number of samples in group	New method	Average count by Wildman method	Range of Wildman counts
	<i>mm. per mgm.</i>	<i>per cent positive fields</i>	<i>per cent positive fields</i>
9	1-5	10	0-16
5	5-10	33	12-46
12	10-15	49	32-68
9	15-20	69	49-80
8	20-25	80	64-96
3	25-35	87	76-96
5	35-50	93	88-96
1	75.01	96	.....

and a Wildman count of 32 and the second 12.21 mm. per mgm. and 68 per cent Wildman count. Similarly two samples with about 16 mm. per mgm. total mold, gave Wildman counts of 49 and 80 respectively.

#### DISCUSSION

The success in duplicating results from one sample or operator to the next emphasizes the quantitative nature of the new method. In order for the method to be actually quantitative and consistent, however, it is necessary that the operator be thoroughly familiar with microscopic appearance of mold fragments and also make absolutely certain that he is accurately estimating lengths of mold fragments in terms of mm. The ruled ocular disc employed for the Howard or Wildman counts provides sufficient rulings to enable a reasonably accurate estimation of total combined length of mold fragments in every field. Once the operator has become accustomed to estimating length of filaments, the method becomes almost as rapid as that of Wildman.

The method as it exists has some advantages and disadvantages. It does provide a truer picture of the actual mold hyphae content than any other method. The results of Claydon indicate that the degree of working in the churn affects the number and length of mold filaments in the final butter and consequently the Wildman mold mycelia value. By the new method all filaments are included whether long or short. Neither the Wildman nor the new method includes the tiniest fragments of *Oospora lactis* hyphae sometimes referred to as spores. However, examination indicated that only a comparatively small number of the tiny fragments remain in the butter. Most of them pass out into buttermilk and wash water.

There is a definite advantage to knowing the actual total mold content of a sample. The commercial samples in these studies running as high as 49 and 75 mm. per mgm. total mold filament provide an example of a problem confronting many creameries. The high temperatures and relative humidities in this part of the country are quite conducive to mold growth in cream on the farm. Creameries in this area are therefore blessed with an abundance of mold growth in the cream during the warm season. The result is that much 90 score butter runs over 60 per cent by the Wildman test. The 89 score butter is often correspondingly higher in mold content. Much of it runs as high as 90 to 100 by the Wildman test. Such butter may contain as much as 75 mm. per mgm. of mold by the new method. If the creameryman in a quality improvement program eliminates half the moldy cream, he may bring the mold content of his butter down to about 40-50 mm. Yet it still will run 90 or over by the Wildman test. If he then again eliminates 50 per cent of this moldy cream, he reduces the mold content of his butter to perhaps 25 mm. per mgm. Again instead of reducing the Wildman count 50 per cent he may end up with a 90 to 100 per cent mold mycelia count. It is not until the mold content is reduced below 20-25 mm. per mgm. that a distinct lowering in mold content of butter can be detected by the Wildman mold mycelia count. This condition has been responsible for considerable misunderstanding in cream quality improvement work.

The variation in Wildman mold mycelia count values for butters of equivalent total mold content indicates that more study should be given to factors possibly responsible for such variations. A variation of 20 or 25 per cent due possibly to manufacturing conditions may easily throw a butter from a definitely legal into an illegal class.

Another advantage of the new method is that any microscope of any field diameter can be employed as long as it provides about 100 $\times$  magnification and a stage micrometer is available to determine the field diameter. Also, a permanent preparation of the sample is obtained which can be examined weeks later if more convenient. Materials other than butter can be diluted in hot gum solution and their total content of mold filament determined. Because of the interfering effect of the protein, samples of cream and butter-

milk must be diluted out further than butter. Because of its low mold content, butter wash water must be diluted less than butter. Covering the preparation with immersion oil is especially helpful in examining cream and buttermilk preparations. In cream examination, special methods are necessary to thoroughly agitate the samples in order to break up masses of mold filaments.

This method was not developed nor is it presented for the purpose of replacing the Wildman count. It may appear too technical for the average control laboratory. However, it has been about as easy to teach to a careful operator as the Wildman method. The new method, because it is quantitative, has proven a valuable research tool. Investigations on mold content of butter and other materials have been carried out that would not have been possible by any other method.

#### SUMMARY

A method for determination of total combined length of dead mold fragments per gram or milligram of butter has been developed.

The procedure consists of dilution of one gram of butter in hot gum solution, spreading a small quantity of this on a glass slide, staining with Newman's stain and examining with low power magnification. The average length of mold filament per field is multiplied by the microscope factor divided by 1000 and results reported in mm. per mgm. of butter.

This quantitative method has been of value in studies to determine the total mold fragment content of butter, buttermilk, wash water and cream.

#### ACKNOWLEDGMENT

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# THE DETERMINATION OF CITRIC ACID IN MILK PRODUCTS BY CERATE OXIDIMETRY

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Of the various methods which have been proposed for the determination of citric acid in milk, those based on Stahre's (12) reaction have come into wide use. In this method, the citric acid is oxidized by dilute potassium permanganate in the presence of bromine resulting in the formation of insoluble pentabromacetone, which is determined gravimetrically.

Allen (1) points out three objections to this method: "1), the formation of pentabromacetone as an oil instead of a crystalline precipitate; 2), the relatively high solubility of the precipitate; 3), the loss due to volatility when moist."

By determining the solubility of the precipitate (and using a factor to include this loss in the final calculations) and by drying the precipitate in a vacuum at less than 20° C. for sixteen hours, Deysher and Holm (2) largely eliminated the second and third errors pointed out by Allen. But in so doing, the length of time of performing the test was extended another twenty-four hours, making a total length of time of three days.

If the determination of citric acid is to be used as a control test in the manufacture of dairy products, particularly evaporated milk, a much shorter method is necessary.

A search of literature, therefore, was made to ascertain if other methods would be applicable. The conductimetric method of Kolthoff (6) was tried but found to be unsuitable for milk owing to the high concentration of other ions, particularly chloride. Potentiometric titrations using silver nitrate, barium acetate, and lead acetate resulted in failures because of interfering phosphate and chloride ions. Rundell (9) states that attempts to determine citric acid potentiometrically with calcium chloride were unsuccessful.

The iodimetric method of Kometianni (7) involves precipitation of the citric acid as pentabromacetone under conditions which are not in accordance with those found necessary by Deysher and Holm for quantitative recovery. This is in agreement with Templeton's (13) statement that the iodimetric method is not quantitative. Hartman (3) also states that Kometianni's procedure is not entirely satisfactory when applied to mixed fruit acids. If the method were modified to insure accuracy, it would offer no advantage over the Deysher and Holm procedure.

Colorimetric methods in which acetic anhydride and pyridine are used to develop color directly from the citrate ion were found to be unsatisfactory, probably due to the presence of the chloride ion. Methods using

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acetone dicarboxylic acid or pentabromacetone for a basis for color development were not tried since no great saving in time would have resulted.

Willard and Young (14) showed that ceric sulphate in sulphuric acid solutions oxidized citric acid at the rate of 1.211 mg. anhydrous citric acid per 1 ml. of 0.1 N sulphato-cerate. Smith and Duke (10) found that the perchlorato-cerate ion in the presence of perchloric acid oxidized citric acid stoichiometrically at the rate of 1.372 mg. per 1 ml. of 0.1 N perchlorato-cerate. When these two methods were applied to milk, it was found that the former method was not stoichiometric. The following method is based on Hartman and Hillig's procedure (4, 5) for isolating the citric acid (omitting tartaric and phosphotungstic acids which interfere) and Smith and Duke's procedure for oxidizing it. In preliminary work, to determine the adaptability of their indicator to the method as applied to milk, a Beckman pH set was employed using a glass electrode as reference electrode and polished platinum wire as indicating electrode following the suggestion of Lykken (8).

#### PREPARATION OF REAGENTS

1. *Ammonium perchlorato-cerate*. Smith (11). Dissolve 55 to 56 grams of  $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$  by adding the salt to a liter beaker containing 340 to 345 ml. of 72 per cent perchloric acid. Stir well during a half-minute interval and add 100 ml. of water. Again stir well for a half-minute interval and add an additional 100 ml. of water. Continue this procedure until the volume attains 1 liter. Transfer to a suitable glass-stoppered reagent bottle and store in the dark. It is preferable to store the perchlorato-cerate solution in a black bottle, conveniently made by completely covering a reagent bottle with black electrical insulating tape. Such a solution requires standardization about every fifteen days. Carbon or dust if allowed to come in contact with perchlorato-cerate solutions catalyze their slight deterioration rate. The perchlorato-cerate solution is standardized by measuring 20.00 ml. into a 150 ml. beaker and adding 15 ml. of 70 per cent perchloric acid, 35 ml. of water, and 1 drop of nitro-ferroin. Titrate with 0.1 N sodium oxalate to the first formation of a pink color.

2. *Sodium oxalate in 0.1 N perchloric acid*. Smith (11). Dissolve 6.706 grams of Bureau of Standards, standard of reference sodium oxalate, per liter of 0.1 N perchloric acid. This solution is stable upon storage. It is used to standardize the ammonium perchlorato-cerate solution and to titrate the excess perchlorato-cerate left after oxidation of the citric acid.

3. *Nitro-ferroin* (nitro-o-phenanthroline ferrous complex) used as internal indicator is commercially available.

4. *Lead acetate*. Dissolve 75 gms. of normal lead acetate in water, add 1 ml. of glacial acetic acid and dilute to 250 ml.

5. *Blank solution*. Dissolve 0.5 gm. lactose, 0.2 gm.  $\text{CaCl}_2$ , and 0.2 gm.  $\text{KH}_2\text{PO}_4$  in 100 ml.  $\text{H}_2\text{O}$ .

6. *Saturated lead citrate.* Add a slight excess of the lead acetate solution to a 10 per cent solution of A.C.S. grade citric acid in distilled water. Wash several times with distilled water and allow to stand at least twenty-four hours. Filter through No. 42 Whatman or similar grade filter paper before using. (At 25° C., lead citrate is soluble to the extent of 6 mg. per 100 ml. of water.)

#### PROCEDURE

Weigh 50 grams of whole milk, 25 grams of evaporated milk, or 5 grams of milk powder in a 150-ml. beaker. Add 25 ml. of water to the evaporated milk and 45 ml. to the dried milk. Add 6 ml. of 1 N sulphuric acid and heat for fifteen minutes on a steam bath. Cool. Add 95 per cent ethyl alcohol and transfer to a 250-ml. volumetric flask. Make to 250 ml. with alcohol. Mix and filter through a good grade of filter paper (such as Whatman No. 1). If filtrate is not clear, repeat the filtration. Place 100.0 ml. of the filtrate in a 100-ml. centrifuge tube and add 4 ml. of lead acetate solution. Allow to stand 3–5 minutes with occasional shaking. Centrifuge 5 minutes. Decant, testing the alcoholic solution with a few drops of lead acetate to insure complete recovery of lead citrate. Drain for 5 minutes. Add 30 ml. of water saturated with lead citrate. Shake until precipitate is dislodged, centrifuge 5 minutes, decant, and drain for 5 minutes. Wash 2 more times with 20-ml. portions of water saturated with lead citrate, centrifuging and draining 5 minutes each time. Add 10 ml. water and shake to dislodge the precipitate. Transfer to a 250-ml. beaker using a total of 55 ml. of 70 per cent perchloric acid to wash the centrifuge tube, pouring the washings into the beaker. Place beaker in ice and salt mixture until temperature reaches – 3° to 0° C. Add 40.0 ml. 0.1 N perchlorato-cerate slowly with stirring, not allowing the temperature in the beaker to rise above 10° C. Hold for 30 minutes at 10° C. Add 95 ml. water, 2 drops of nitro-ferroin and titrate with 0.1 N sodium oxalate.

A blank determination must be run with each group as follows:

Place 25 ml. of the prepared blank solution in a 150 ml. beaker. Add 3 ml. H<sub>2</sub>SO<sub>4</sub>. Heat in steam bath with other samples. Cool. Add 95 per cent alcohol and make to 125 ml. Mix and filter. Continue exactly as described for milk, except that at the conclusion of the final draining period, 30 ml. of water are added and the contents of the centrifuge tube transferred to a 250-ml. beaker with 55 ml. 70 per cent perchloric acid. When the temperature has reached – 3° to 0° C., 10.00 ml. of 0.1 N perchlorato-cerate are added and the solution held for 30 minutes at 10° C. Add 95 ml. water, 2 drops of nitro-ferroin and titrate with 0.1 N oxalate. The ml. of 0.1 N cerate used constitute the blank.

Per cent citric acid (anhydrous)

$$= \frac{\text{ml. 0.1N perchlorato-cerate used} - \text{blank} \times 0.343}{\text{wt. sample}}$$

TABLE 1

*Recovery of citric acid from pure solutions\**

Citric acid taken	Recovered	Recovered
mg.	mg.	%
33.51	33.68	100.52
33.51	33.23	99.17
33.51	33.71	100.59
33.57	33.57	100.00
33.57	33.63	100.18
33.57	33.42	99.55

\* 1 ml. 0.1 N perchlorato-cerate is equivalent to 1.372 mg. anhydrous citric acid.

The potentiometric method for the detection of the endpoint is equally as satisfactory as the use of nitro-ferroin as an internal indicator. The results included in this paper were secured with the nitro-ferroin indicator.

## EXPERIMENTAL

Various quantities of anhydrous citric acid (prepared by grinding to a powder and drying Baker's analyzed and Merck's A.C.S. grade over calcium chloride) were dissolved in distilled water. Sufficient 70 per cent perchloric acid was added to make the solution 4 M with respect to the perchloric acid. After cooling the mixture to 0° C., a slight excess of 0.1 N perchlorato-cerate was added and the solution held at 10° C. for 30 minutes. It was then diluted to 2 N and titrated with 0.1 N sodium oxalate using nitro-ferroin as the indicator. The results given in table 1 show an average recovery of 100.00 per cent. It was found that if the temperature rose above 12° C., the results were high owing to side reactions as pointed out by Smith (11).

Four cultures of citric acid fermenting bacteria were secured from Iowa State College. These were *S. diacetilactis* strain N 4, and *S. citravorous* strains S 9, S 14, and Mu 29. These were inoculated into acidified sterile skim milk and incubated until citric acid was absent as shown by the penta-bromacetone determination. Volumetric citrate determinations were made

TABLE 2

*Recovery of citric acid from decitrated milk*

Citric acid taken	Recovered	Recovered
mg.	mg.	%
15.1	15.1	100.0
22.2	22.8	102.7
42.4	43.3	102.1
45.4	46.0	101.3
69.3	70.0	101.0
90.0	90.1	100.1
98.9	99.0	100.1
98.9	98.8	99.9



on these cultures with known quantities of anhydrous citric acid added. These results are given in table 2. The average percentage recovery is 100.9 per cent.

It was found that decitrated milk gave a larger blank value than when the procedure was followed using only distilled water. It was thought that this might be due to some interfering substance in milk or to the formation of some interfering substance during the growth of the citric-acid-fermenting bacteria. Various natural components of milk were tested, including lactose, riboflavin, niacin, ascorbic acid, calcium and magnesium salts, phosphates, chlorides, nicotinic acid, pantothenic acid, lactic acid, propionic acid, butyric acid, acetylmethylcarbinol, and ether extract from milk but none of these was found to interfere. It was found that when the volume of the precipitate

TABLE 3

Method of preparation of blank	ml. of 0.1 N perchlorato-cerate required per 100 ml. filtrate
Fermentation of citric acid	2.35
	2.45
	3.11
	2.38
	3.27
	2.88
Precipitation of proteins and citrate with silver acetate and acetic acid	2.60
	2.94
	2.68
	1.57
Prepared blank (containing lactose, Ca, Cl, $\text{PO}_4$ )	2.10
	2.24
	3.35
	2.87
	3.35

was the same as that obtained from milk the blank was about the same. Since repeated washings of the precipitate did not appreciably reduce the value of the blank it was thought that possibly a small amount of the lactose was occluded in the precipitation of lead citrate. However, when the thrice washed precipitate was tested for lactose using copper sulphate and alkaline tartrate solutions, no observable precipitate of copper oxide was formed. Furthermore, when perchloric acid is added to the washed precipitate of lead salts, both in the case of the decitrated milk and in the case of the various milk products examined, a yellow color is formed which does not form with any of the substances added to the blank. It is apparent, therefore, that some substances not included in this study is present in the lead salt precipitate which may interfere.

Another method for the preparation of the blank was tried, using 0.5 gm. silver acetate and 5 ml. of 10 per cent acetic acid per 50 ml. of milk in place of 6 ml.  $\text{NH}_2\text{SO}_4$ . By this procedure the citrate is precipitated as silver

citrate and filtered off. To 100 ml. of the filtrate, 4 ml. of lead acetate were added and the precipitate washed as previously described. This precipitate did not give the color reaction with perchloric acid and the blanks were slightly lower than those from the decitrated milk. However, the volume of the precipitated lead salts is small. A resume of these results is given in table 3.

Table 4 gives results of some analyses of powdered skim milk, whole milk, and evaporated milk. The volumetric citrate method gives results which are slightly higher due either to an interfering substance in milk or incomplete recovery by the pentabromacetone method.

TABLE 4

Product		Oxidation by perchlorato-cerate	Pentabromacetone (Modification of Deysher and Holm)
Powdered milk:	(1)	1.57	1.55
		1.57	1.52
	(2)	1.82	1.67
		1.87	1.63
	(3)	1.82	1.78
		1.80	1.74
Whole milk:	(1)	0.172	0.161
		0.176	0.168
	(2)	0.178	0.159
		0.168	0.167
	(3)	0.171	0.165
		0.171	0.160
Evaporated milk:	(1)	0.332	0.323
		0.325	0.327
	(2)	0.334	0.330
		0.340	0.333
	(3)	0.342	0.329
		0.333	0.338

## CONCLUSIONS

A method for the determination of citric acid in milk products is presented which is based on the oxidation of citric acid (isolated from interfering substances by precipitation as lead citrate) by an excess of 0.1 N perchlorato-cerate in 4 M perchloric acid solution. The excess perchlorato-cerate is titrated with 0.1 N sodium-oxalate in 2 M perchloric acid using nitro-ferroin as an internal indicator.

Care must be used to free the citric acid from other organic substances which are also oxidized by the perchlorato-cerate. The results from the method are slightly higher than those from the pentabromacetone method and can be secured in considerably less time.

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# THE EFFECT OF IODINATED CASEIN (PROTAMONE) ON MILK AND BUTTERFAT PRODUCTION AND ON THE ASCORBIC ACID CONTENT OF THE MILK\*

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In 1934, Graham (3) reported the results of extensive studies with thyroidectomized dairy cattle and with the feeding of desiccated thyroid glands to lactating cows. In this experiment the diminution in milk secretion following the removal of the thyroid could not be distinguished readily from that accompanying a control operation without the removal of the thyroid gland; however, there was a distinct rise in milk secretion, when small amounts of dried thyroid glands were fed either to thyroidectomized cows or to unoperated normal cows in the declining phases of lactation. The stimulating factor in the thyroid glands was shown to be thyroxine by an increase in milk secretion when synthetic thyroxine was injected (4). These observations have since been confirmed and extended by Jack and Bechdel (7), Folley and White (2), and Herman, Graham and Turner (5).

The practical value of feeding pure thyroxine or desiccated thyroid glands to increase milk production was largely nullified by the exorbitant cost of this material. In 1940, Turner (13) reported a possible cheap source of material with thyroxine activity in the form of iodinated casein. Reineke and Turner (10) have recently described a method for the preparation and the use of this synthetic thyroprotein. This material is prepared by the iodination of casein in solution to which sufficient sodium bicarbonate has been added to maintain the pH of the reaction mixture within the range of 6.8 to 8.0.

The administration of this artificial thyroprotein to cows and goats, according to Reineke and Turner (11), stimulates increases in milk production and the percentage of fat in the milk similar in every respect to those produced by either desiccated thyroid glands or synthetic thyroxine.

The purpose of this paper is to report the results obtained with lactating dairy cows in the declining part of the lactation period, when the iodinated casein was fed in smaller quantities but for longer periods of time than that reported previously by Reineke and Turner (11).

## EXPERIMENTAL

Six pure-bred Holstein cows in the sixth to the eighth month of their first lactation were selected from the regular milking herd for this experiment.

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The cows were producing from 30 to 40 pounds of milk per day at the beginning of the experiment. The experiment was a double reversal feeding trial started on the twenty-first day of February and continued for a period of 13 weeks or until May 22. The first week was used as an adjustment period at which time the cows were divided into two equal groups of three cows each on the basis of milk production, fat production, and body weight.

Group 1, composed of cows number 535, 536, and 544 was fed 15 grams of iodinated casein (Protamone) per cow daily as a supplement to the regular ration for the first 4-week period. The iodinated casein was discontinued for the second 4-week period after which it was again fed during the third 4-week period.

Group 2, consisting of cows number 527, 529, and 541, served as a check for the first 4-week period and then was fed 15 grams of the iodinated casein per cow daily for the second 4-week period, after which it was discontinued for the last or the third 4-week period.

During the first two 4-week periods, the cows were confined to the barn except when they were allowed to run outside in a clean lot for exercise. During the last 4-week period of the experiment the cows were on pasture. The ration fed the cows in each group was composed of a concentrate mixture, legume hay and beet pulp. The cows were fed daily 10 pounds of concentrates, 2 pounds beet pulp and all the hay they would clean up. They were fed a uniform amount of feed throughout the experiment regardless of the amount of milk produced and the gain or loss in body weight.

The cows were milked twice daily. On Monday, Wednesday, and Friday of each week they were weighed and one-half pint samples of milk were collected night and morning for analysis. Each sample of milk was analyzed for fat by the Babcock method and for total solids by the oven method. Ascorbic acid was titrated in the samples collected each morning with 2-6 dichlorophenolindophenol as described by Sharp (12).

Close observations were made each day for any unusual reaction which might become apparent. A maximum-minimum thermometer placed in the barn was read daily in order to note any unusual change in temperature. Respiration and pulse rates were obtained between 2 and 4 P.M. on Friday of each week during the first two 4-week periods when the cows were confined to the barn. The pulse was counted by feeling the posterior tibial artery on the medial surface of the tibia at a point eight to ten inches above the hock joint.

#### RESULTS

Data showing the effect of feeding iodinated casein on milk and butterfat production; the percentage fat and solids-not-fat; the ascorbic acid in the milk and changes in body weight are presented in table 1 and in figures 1 to 6. Data for individual days were averaged and presented as weekly averages for individual animals or as groups as the case might be. The

figures represent averages for the separate groups except in the case of figure 4, which shows the effect of iodinated casein on solids-not-fat and where variation in the results obtained made it seem desirable to present data for individual animals. It may be observed in table 1, that data for 529 are incomplete. This animal appeared normal until about the middle of the second 4-week feeding period. She had received 15 grams of the iodinated casein daily for two weeks when she began to show the symptoms of a rabid animal. She refused to eat and lost in body weight rapidly, and died in about a week after showing the first symptoms. After an examination of the brain, the cause of the death of the animal was definitely diagnosed as rabies. Data from this animal are not included in the averages for Group 2.

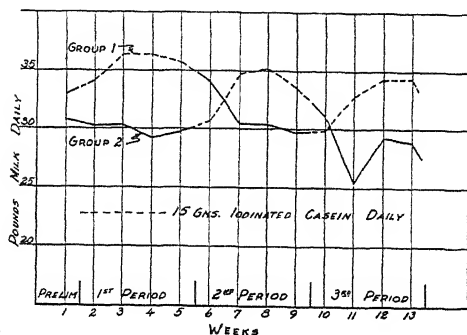


FIG. 1. Effect of iodinated casein on milk production.

*Effect on milk production.* Data presented in table 1 and in figure 1 show that the feeding of 15 grams of iodinated casein daily, as a supplement to the regular ration, did cause a definite increase in the milk production of all animals on experiment. It may be observed in figure 1 that the cows increased in milk production for about two or three weeks after the beginning of iodinated casein feeding, after which there was a tendency to show a slight downward trend. It may be observed in the case of the cows in Group 2 that milk production showed a sharp decline for about two weeks during the third period when iodinated casein had been discontinued. During the latter part of the third period there was an increase in milk production which is thought to be due to the cows going out on pasture. At that particular time the cows in Group 1 were receiving iodinated casein which probably supplemented the stimulating effect of pasture.

*Effect on percentage fat content of the milk.* Data summarized in figure 2, show the effect of feeding iodinated casein on the fat content of the milk. The cows in both groups showed an increase in percentage fat content of the milk during the feeding periods. It may be observed that there was considerable carry-over effect following the feeding of the iodinated casein. After the feeding of the iodinated casein, the cows continued to secrete milk





TABLE 1—(Continued)

Weeks	Date	Milk, lbs.	Fat, %	Fat, lbs.	S-N-F, %	Ascorbic acid, mg./liter	Milk, lbs.	Fat, %	Fat, lbs.	S-N-F, %	Ascorbic acid, mg./liter	Milk, lbs.	Fat, %	Fat, lbs.	S-N-F, %	Ascorbic acid, mg./liter
Group II																
15 grams of iodinated casein daily																
		Cow Number 527					Cow Number 529					Cow Number 541				
1	Feb. 21-27	34.6	3.77	1.30	8.36	17.2	33.0	3.87	1.28	8.53	19.7	27.0	3.25	0.88	8.51	19.0
2	Feb. 28-Mar. 6	34.4	3.88	1.33	8.30	17.0	31.1	3.78	1.18	8.63	19.5	25.9	3.76	0.97	8.41	19.9
3	Mar. 7-13	34.4	3.77	1.30	8.33	17.7	32.9	3.77	1.24	8.62	19.4	26.1	3.52	0.92	8.56	19.3
4	Mar. 14-20	32.6	3.83	1.25	8.15	15.9	31.1	3.98	1.24	8.60	19.0	25.9	3.52	0.91	8.45	18.0
5	Mar. 21-27	33.8	3.85	1.30	8.25	15.8	30.2	4.03	1.22	8.69	18.7	25.7	3.67	0.94	8.55	17.7
Iodinated casein discontinued, cows on pasture																
6	Mar. 28-Apr. 3	34.4	3.81	1.31	8.17	12.7	32.9	3.69	1.21	8.68	11.9	27.0	3.46	0.93	8.72	16.6
7	Apr. 4-10	38.6	4.32	1.67	8.39	9.6	35.3	3.50	1.24	8.77	13.8	31.1	3.60	1.12	8.64	12.1
8	Apr. 11-17	39.1	4.21	1.65	8.99	8.6	.....*	.....	.....	.....	.....	31.3	3.97	1.24	9.10	12.2
9	Apr. 18-24	36.3	4.19	1.52	8.76	10.0	.....	.....	.....	.....	.....	31.1	4.52	1.41	8.89	13.5
10	Apr. 25-May 1	32.8	4.12	1.35	8.89	13.3	.....	.....	.....	.....	.....	29.4	3.93	1.16	8.94	13.2
11	May 2-8	27.3	4.64	1.27	8.67	15.5	.....	.....	.....	.....	.....	23.3	4.34	1.01	8.81	16.2
12	May 9-15	32.4	4.32	1.40	8.35	13.1	.....	.....	.....	.....	.....	26.2	4.71	1.23	8.39	14.4
13	May 16-22	31.7	4.06	1.29	8.39	14.1	.....	.....	.....	.....	.....	26.2	4.28	1.12	8.33	12.8

\* Cow died with rabies.

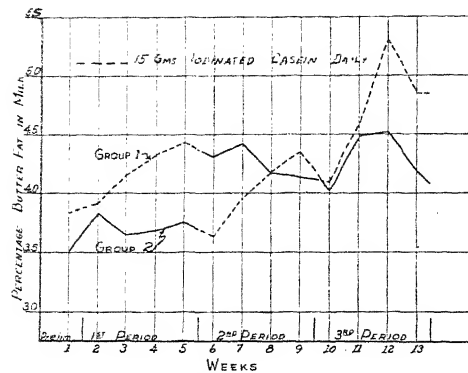


FIG. 2. Effect of iodinated casein on percentage butterfat in milk.

of a higher fat content than before, for a period of at least four weeks. In the case of the cows in Group 1, there was an increase from an average of about 3.8 per cent to about 4.4 at the end of the first 4-week feeding period. During the next 4-week period when the cows did not receive the iodinated casein the percentage fat decreased only about 0.2 per cent. During the last 4-week feeding period when the cows were again fed iodinated casein, the fat percentage increased to about 5 per cent. This represents about 1.2 per cent increase in the fat content of the milk during the experiment. A small part of this increase in fat percentage may have been due to advance in lactation.

The cows in Group 2 showed an increase in the percentage fat from about 3.7 to about 4.4 per cent after four weeks of casein feeding. They were able to maintain a level of about 4.4 per cent for the following four weeks when they received no iodinated casein.

*Effect on total fat production.* Data summarized in figure 3, show the effect of iodinated casein on total fat production. In general butterfat pro-

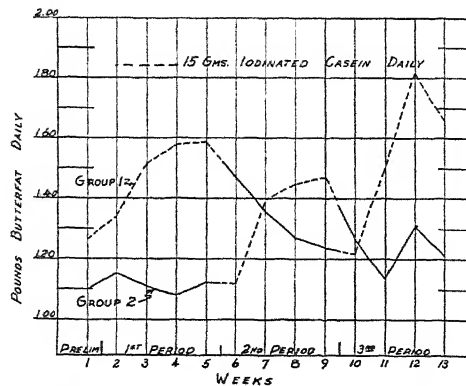


FIG. 3. Effect of iodinated casein on butterfat production.

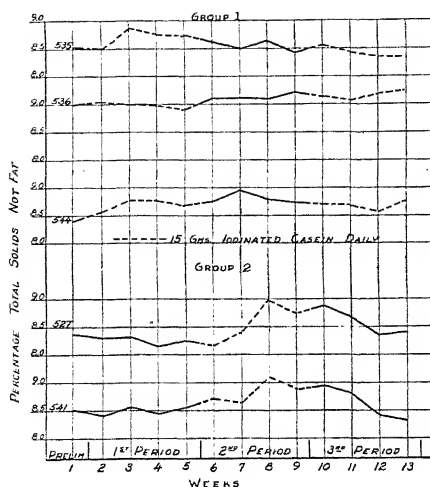


FIG. 4. Effect of iodinated casein on the percentage total solids-not-fat of milk.

duction followed about the same trend as milk production for the animals in both groups. The relative change in fat production due to casein feeding was considerably greater than in the case of milk production. This was brought about by both an increase in milk production and an increase in the percentage fat content of the milk.

*Effect on the solids-not-fat content of the milk.* In table 1 and in figure 4 are presented data for the solids-not-fat content of the milk for the animals in both groups. It may be observed that the changes in the solids-not-fat content are small, but there seems to be a tendency for a small increase for animals Number 535 and 544 during the first iodinated casein feeding periods, with a slight decrease during the next period for Number 535 when she did not receive iodinated casein. In the case of cows Number 527 and

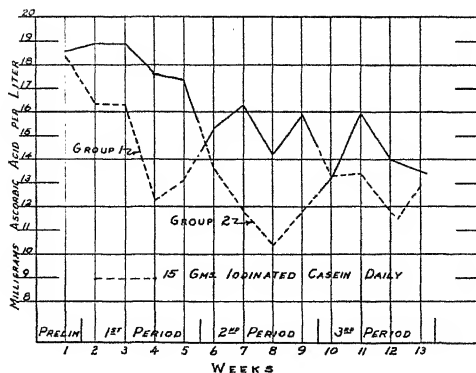


FIG. 5. Effect of iodinated casein on ascorbic acid content of milk.

541, there seems to be a much more definite tendency for the solids-not-fat to show an increase during the iodinated casein feeding with a decrease during the next 4-week period when the cows did not receive any iodinated casein.

*Effect on the ascorbic acid content of the milk.* In figure 5 is presented a summary of the data presented in table 1, showing the effect of feeding iodinated casein on the ascorbic acid content of the milk. It may be observed that there was a definite decrease in the ascorbic acid content of the milk in all cases when iodinated casein was fed. When the iodinated casein feeding was discontinued there was an increase in the ascorbic acid in the milk, but there seemed to be some carry-over effect. It may be observed that the general trend was downward as the experiment progressed.

*Effect on change in body weight of the cows.* In figure 6 is presented a summary of the changes in body weight of the cows in both groups. It may

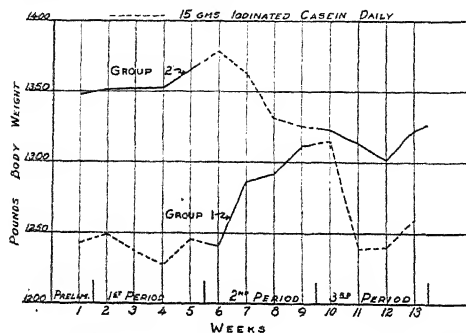


FIG. 6. Effect of iodinated casein on body weight.

be noted that the cows in Group 1 did not show any particular change in body weight during the first iodinated casein feeding period but showed an increase of about seventy pounds during the second 4-week period when iodinated casein was not fed. During the third period when they received iodinated casein they showed a decrease in body weight which was about equal to the gain made during the preceding period.

The cows in Group 2 showed a tendency to gain slightly in body weight during the first 4-week period when not receiving iodinated casein but declined considerably during the second 4-week period when they received iodinated casein. They continued to show a decline in body weight for about two weeks during the third period when they were not receiving iodinated casein. After the middle of the third period body weight began showing an upward trend when they were turned to pasture.

*Effect on pulse rate and respiration.* In table 2 are presented data showing the effect of iodinated casein on the pulse and respiration rates. Data are also presented showing the minimum and maximum barn temperatures on the days that the pulse and respiration rates were determined.

TABLE 2  
*Effect of iodinated casein on the pulse rate and respiration*

	Group 1								Group 2								Barn tem- perature, °F.												
	535				544				Average				527						529				541				Average		
	Pulse		Resp.		Pulse		Resp.		Pulse		Resp.		Pulse		Resp.				Pulse		Resp.		Pulse		Resp.		Pulse		Resp.
																													Min.
Feb. 23 .....	69	22	70	26	68	28	69	25	65	25	62	26	68	24	65	25	58	67											
Feb. 26 .....	62	18	69	16	57	18	63	17	71	16	58	20	57	16	62	17	40	46											
15 grams iodinated casein daily																													
Mar. 5 .....	72	20	80	24	68	21	73	22	62	17	65	16	63	22	64	18	42	54											
Mar. 11 .....	76	28	80	30	68	28	75	29	60	15	64	17	62	20	62	17	45	57											
Mar. 20 .....	76	22	72	20	74	25	74	22	64	18	65	16	64	22	64	19	48	54											
Mar. 26 .....	76	22	84	30	84	33	81	28	62	22	60	22	64	28	62	24	54	65											
Iodinated casein discontinued																													
Apr. 2 .....	64	18	72	25	76	26	71	23	72	26	80	33	68	27	73	29	43	66											
Apr. 9 .....	64	24	64	32	72	36	67	31	80	32	68	25	72	24	73	27	54	70											
Apr. 16 .....	64	18	60	22	72	35	65	25	72	27	.....†	.....	68	24	68	25	44	60											
Apr. 23 .....	60	28	64	25	68	28	64	27	80	35	.....	.....	72	36	76	36	55	71											
15 grams iodinated casein daily																													
Iodinated casein discontinued																													
Apr. 30 .....	64	25	72	30	72	42	69	32	64	27	.....	.....	64	25	64	26	43	72											
May 7* .....	80	50	88	60	72	50	80	53	72	24	.....	.....	64	40	68	32	60	80											

\* Cows were on pasture during forenoon.  
† Animal died with rabies Data not included in average for group.

It may be observed that there was considerable variation in the pulse rate and respiration rate for the cows in the two groups, nevertheless there was a definite tendency for an increase in pulse rate and respiration with iodinated casein feeding. There was also an increase in respiration with an increase in the barn temperature as might be expected.

#### DISCUSSION

The results obtained in this experiment show clearly that iodinated casein will stimulate an increase in milk production and the percentage fat in the milk. There is some indication of a slight increase in the percentage solids-not-fat and a considerable decrease in the ascorbic acid content of the milk.

The effect on milk production and the composition of the milk was observed after about 4 to 5 days of iodinated casein feeding. Reineke and Turner (11) observed an increase in milk production after the third day when they were feeding from 50 to 100 grams daily. Cows in the declining phase of lactation fed 15 grams of iodinated casein daily (7.5 grams morning and evening) for a period of four weeks showed an increase of from 5 to 20 per cent in milk production and from 25 to 50 per cent in butterfat production.

The increase in butter fat production was due to both an increase in milk production and an increase in the percentage fat content of the milk. There was considerable carry-over effect from one 4-week period to another in the percentage fat content of the milk. There is some evidence that the cows did not reach the peak for fat percentage of the milk in the 4-week feeding periods. It may be seen that the cows in Group 1 continued to rise in the percentage fat of the milk for the second 4-week iodinated casein feeding period. The butter fat percentage appeared to be influenced more by iodinated casein feeding than milk production.

The solids-not-fat content of the milk of cows fed iodinated casein was only slightly increased which was probably not significant. Herman, Graham and Turner (6) obtained only a slight increase in the solids-not-fat content of the milk when thyroxine was injected subcutaneously or when desiccated thyroid was fed orally.

The feeding of 15 grams of iodinated casein daily was sufficient to cause a decrease of approximately thirty-three per cent in the ascorbic acid content of the milk. This is very similar to results reported earlier by Brown, Van Landingham and Weakley (1) when potassium iodide was fed. When fed at the rate of 5 grams daily for two weeks postassium iodide did not cause an increase in the rate of milk production. What effect, if any, iodinated casein may have on the ascorbic acid content of the blood is yet to be determined. Nevertheless one might expect a reduction in the ascorbic acid content of the blood corresponding to the reduction in the ascorbic acid content

of the milk. Recent investigations have shown that the level of ascorbic acid in the blood plasma is closely associated with fertility in dairy cattle. Results obtained by Philips *et al.* (8, 9) with ascorbacidothrapy on sterile bulls and "hard to settle" cows indicate that ascorbic acid is an important factor in breeding efficiency.

Data obtained on the effect of feeding iodinated casein on respiration and pulse rate are very limited, nevertheless there was a tendency for the rate of respiration to increase. There was also an increase of about 10 beats per minute in pulse rate when iodinated casein was fed. This seems to be in line with observations of Reineke and Turner (11).

Changes in body weight were noted but probably did not affect the animal adversely in the short time covered by this experiment.

#### SUMMARY

The effect of feeding 15 grams of iodinated casein (Protamone) to milking cows in the declining part of their first lactation has been studied and the following results obtained:

1. Changes in milk production and in the composition of the milk were apparent after about four to five days of iodinated casein feeding.

2. Cows in the declining part of lactation showed an increase of 5 to 20 per cent in milk production and from 25 to 50 per cent in butterfat production.

3. During the first four-weeks' feeding of iodinated casein the fat content of the milk was increased by 0.47 to 0.98 per cent above the fat content of the milk at the beginning of the experiment. When iodinated casein was discontinued for four weeks and then fed for a second four-week period, the fat content was increased by 0.90 to 2.03 per cent above the fat content of the milk at the beginning of the experiment.

4. There was only a slight increase in the solids-not-fat content of the milk.

5. There was a decrease of about thirty-three per cent in ascorbic acid content of the milk.

6. There was also an increase in respiration and pulse rate, and a small decrease in body weight.

#### ACKNOWLEDGMENT

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- (11) REINEKE, E. P., AND TURNER, C. W. Increased Milk and Fat Production Following the Feeding of Artificially Formed Thyroproteins (Thyrolactin). *JOUR. DAIRY SCI.*, 25: 393. 1942.
- (12) SHARP, PAUL F. Rapid Method for the Quantitative Determination of Reduced Ascorbic Acid in Milk. *JOUR. DAIRY SCI.*, 21: 85. 1938.
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PROGRAM  
THIRTY-NINTH ANNUAL MEETING  
OF THE  
AMERICAN DAIRY SCIENCE ASSOCIATION  
OHIO STATE UNIVERSITY  
COLUMBUS, OHIO  
JUNE 20-22, 1944

PROGRAM COMMITTEE

GENERAL:

H. P. DAVIS, *Chairman*—University of Nebraska  
DWIGHT ESPE—Iowa State College  
O. F. GARRETT—M & R Dietetic Laboratories  
E. C. SCHEIDENHELM—Michigan State College  
L. H. BURGWARD—Ohio State University

EXTENSION:

E. C. SCHEIDENHELM, *Chairman*—Michigan State College  
FLOYD ARNOLD, University of Maryland  
GERALD HEEBINK—University of West Virginia  
CHARLES L. BLACKMAN—Ohio State University

MANUFACTURING:

O. F. GARRETT, *Chairman*—M & R Dietetic Laboratories  
G. C. NORTH—Illinois  
B. E. HORRALL—Purdue University

PRODUCTION:

DWIGHT ESPE, *Chairman*—Iowa State College  
G. W. SALISBURY—Cornell University  
K. L. TURK—University of Maryland

REGISTRATION

CAMPBELL HALL

OHIO STATE UNIVERSITY

Meetings will be held in buildings on the campus of Ohio State University and headquarters will be there.

COMMITTEE MEETINGS

Suitable rooms will be available for all committees and for other groups which may desire to meet. L. H. Burgwald will have charge of room assignments.

## SCHEDULE OF PROGRAMS

(Eastern War Time)

Date and Time	General	Production Section	Extension Section	Manufacturing Section
<i>Tuesday</i> <i>June 20, 1944</i>				
9:00-12:00	Opening Session	Section A Section B	Section	Section A Section B
1:00- 4:00				
4:00- 5:00	Committees	Committees	Committees	Committees
7:00	Informal Get Together	Symposium		
<i>Wednesday</i> <i>June 21, 1944</i>				
9:00-11:00		Section A Section B	Section	Section A Section B
11:00-12:00		Joint Business Meeting		Business Meeting
1:00- 4:00	Post War Problems in Dairying			
4:00- 5:00	Committees	Committees	Committees	Committees
7:00	Reception			
<i>Thursday</i> <i>June 22, 1944</i>				
9:00-11:00		Joint Symposium		Symposium
11:00-12:00		Business Meeting	Business Meeting	Business Meeting
1:00- 3:30	Latin American			
3:30	Dairying Business Meeting			
7:00	Association Banquet			

## PHOTOGRAPHIC PROJECTION

Projection equipment will be available if required. It is the hope of the committee, however, that tabular and graphic material will be presented in mimeograph form rather than as slides.

## INVITATION TO VISIT OHIO EXPERIMENT STATION

The administration of the Ohio Experiment Station and the members of the Dairy Department staff cordially invite you to visit the Experiment Station at Wooster on your way to or from the Dairy Science meetings. In addition to the regular experimental herd, there is a special herd and farm devoted entirely to pasture research, an experimental forage harvester, and extensive batteries of experimental silos.

Wooster is 90 miles north of Columbus on state routes 3, 5, and 30. It is readily accessible by bus from all directions and by rail from east and west.

## GENERAL PROGRAM

*(Eastern War Time)**Tuesday, June 20, 1944*

9:00-12:00 OPENING SESSION. Campbell Hall, Room 200.

A. C. DAHLBERG, *President, American Dairy Science Association*, Presiding.

Introduction of Officers and Guests.

Address of Welcome—DR. HOWARD L. BEVIS, *President, The Ohio State University*.Response and Presidential Address—A. C. DAHLBERG, *President, American Dairy Science Association*.

Guest Speaker.

Announcements.

1:00- 4:00 SECTIONAL MEETINGS.

Production Section A, Feeds and Feeding, Room 100, B &amp; Z Bldg.

Production Section B, Physiology and Nutrition, Room 209, B &amp; Z Bldg.

Manufacturing Section A, Bacteriology, Room 302, Campbell Hall.

Manufacturing Section B, Chemistry, Room 200, Campbell Hall.

Extension Section, D.H.I.A. work, Room 206, H &amp; F Bldg.

4:00- 5:00 COMMITTEE MEETINGS.

7:00- 9:00 Production Section Symposium, Room 100, B &amp; Z Bldg.

Relation of Body Size to Milk Yield.

A. The Energy-Size Basis of Measuring Milk Yield—W. L. GAINES, *University of Illinois*.B. The Significance of Body Size in Milk Production—SAMUEL BRODY, *University of Missouri*.C. Pertinent Data on the Problem—MAX KLEIBER, *University of California*.D. How the Size Problem Affects Breed Associations—C. T. CONKLIN, *Ayrshire Breeders Association*.7:00 INFORMAL GET TOGETHER—*Faculty Club, Ohio State University*.*Wednesday, June 21, 1944*

9:00-11:00 SECTIONAL MEETINGS.

Production Section A, Feeds and Feeding, Room 100, B &amp; Z Bldg.

Production Section B, Animal Breeding, *Room 209, B & Z Bldg.*

Manufacturing Section A, Butter and Cheese, *Room 203, Campbell Hall.*

Manufacturing Section B, Milk and Other Dairy Products, *Room 200, Campbell Hall.*

Extension Section, *Room 206, H & F Bldg.*

11:00-12:00 BUSINESS MEETINGS OF SECTIONS.

Production and Extension, Joint Business Meeting, *Room 206, H & F Bldg.*

Manufacturing, Business Meeting, *Room 200, Campbell Hall.*

1:00- 4:00 GENERAL SESSION.

Post War Problems of Dairying, *Room 200, Campbell Hall.*

A. Why Different Problems After the War—E. L. ANTHONY, *Michigan State College.*

B. Is Dairy Expansion to Continue?—EARL WEAVER, *Michigan State College.*

C. Improving a Post War Testing Program—C. T. CONKLIN, *Ayrshire Breeders' Association.*

D. Post War Demand for Livestock—O. E. REED, *Bureau of Dairy Industry.*

E. Recommended Nutrient Allowances for Dairy Animals—J. K. LOOSLI, *Cornell University.*

F. Nutrition Education as a Safeguard Against Post War Problems—E. M. HARMON, *National Dairy Council.*

G. Problems of Farm Milk Collection Delivery to Plants and Retail Delivery of Milk—LELAND SPENCER, *Cornell University.*

H. Problems Pertaining to Processing, Manufacturing, and Distribution of Dairy Products in the Post War Period—H. H. SOMMER, LELAND SPENCER, A. W. FARRALL AND R. J. RAMSEY. *A Committee Report.*

4:00- 5:00 COMMITTEE MEETINGS.

7:00 RECEPTION, *Faculty Club.*

*Thursday, June 22, 1944*

9:00-11:00 SECTIONAL MEETINGS.

Manufacturing Section Symposium, *Room 200, Campbell Hall.*

Dehydrated Milk and Milk Products.

A. Types of Dehydrated Milk and Milk Products—P. H. TRACY, *University of Illinois.*

- B. Standard Specifications and Methods of Analysis of Dried Milk—LT. ROBERT REMALEY, *Subsistence Research and Development Laboratory, U. S. Army Quartermaster Corps.*
- C. Use of Anti-oxidants in the Manufacture and Storage of Dried Whole Milk—S. T. COULTER, *University of Minnesota.*
- D. Packaging of Dehydrated Milk and Milk Products—MAJOR JAMES D'A. CLARK, *Subsistence Research and Development Laboratory, U. S. Army Quartermaster Corps.*
- E. The Future of the Dehydrated Milk and Milk Products Industry—J. T. WALSH, *American Dry Milk Institute.*

Production and Extension Symposium, Room 206, H & F Bldg.

#### Mastitis and the Feed Situation.

- A. Mastitis from the Dairyman's Stand Point—T. S. SUTTON, *Ohio State University.*
- B. Modern Methods of Treating Mastitis—C. S. BRYAN, *Michigan State College.*

#### Discussion

- C. The Feed Situation—WALTER BERGER, *Chairman of the Feed and Livestock Division, Food Production Administration.*

#### Discussion

11:00–12:00 Sectional Business Meetings—Production, Room 100, B & Z Bldg.; Manufacturing, Room 200, Campbell Hall; and Extension, Room 206, H & F Bldg.

1:00– 3:30 General Session, Room 200, Campbell Hall.

#### Latin American Dairying.

- A. Dairying and Agriculture—R. E. HODGSON, *Bureau of Dairy Industry.*
- B. Dairy Products in Latin America—A. C. DAHLBERG, *Cornell University.*
- C. Thomas Jefferson, The Farmer—O. E. REED, *Bureau of Dairy Industry.*

3:30 General Business Meeting, Room 200, Campbell Hall.

7:00 Annual Banquet—Installation of Officers and Presentation of Borden Awards.

## SECTIONAL PROGRAMS

## EXTENSION SECTION

*Tuesday, June 20*

Afternoon Session, Room 206, H &amp; F Bldg.

E. C. SCHEIDENHELM—*Chairman*

1:00– 1:15 Opening Business Session.

1:15– 4:00 Sectional Program.

A. Panel Discussion on D.H.I.A. work—*Leader, E. H. LOVELAND, University of Vermont.*E1. Agreement Forms and D.H.I.A. Organization  
—W. T. CRANDALL, *Cornell University.*E2. Short Cuts in Record Keeping—J. F. KENDRICK, *Bureau of Dairy Industry.*E3. A Suggested Form for Permanent and Current Records—J. F. KENDRICK, *Bureau of Dairy Industry.*E4. Securing Supervisors During and Immediately Following the War—C. R. GEARHART, *Pennsylvania State College.*E5. Streamlined Testing for Everybody—G. W. VERGERONT, *University of Wisconsin.*E6. Testing in Areas of Small Herds and Scarce Membership—R. A. CAVE, *South Dakota State College.*B. Report of the Committee—C. R. GEARHART, *Pennsylvania State College.*E7. Wartime 4-H Dairy Club Program—J. C. NAGEOTTE, *Pennsylvania State College.*C. Report of 4-H Club Committee—H. A. WILLMAN, *Cornell University.*

4:00– 5:00 Committee Meetings.

*Wednesday, June 21*

Morning Session, Room 206, H &amp; F Bldg.

E. C. SCHEIDENHELM—*Chairman*

9:00–11:00 Sectional Program.

E8. The Forced Ventilation System of Curing Hay in the Mow—R. G. CONNELLY—*Virginia Polytechnic Institute.*E9. A Complete Sire Proving Program—C. T. CONKLIN, *Ayrshire Breeders Association.*

E10. The Task of Getting Quality Bulls Giving Quality Semen—S. J. BROWNELL, *Cornell University*.

E11. Action Program for Rapid Washing Methods—J. M. JENSEN, *Michigan State College*.

D. Report of Quality and Marketing Committee—E. WALLENFELDT, *University of Wisconsin*.

E. Report of States with Exhibits and Committee on Teaching Ideas—R. T. HARRIS, *University of Wisconsin*.

F. Report of Type Rating Committee—J. W. LINN, *Kansas State College*.

G. Report of Dairy Cattle Health Committee—C. W. REEVES, *University of Tennessee*.

H. Report of Dairy Farm Management Committee—M. J. REGAN, *University of Missouri*.

11:00-12:00 **Joint Business Meeting**—Production and Extension Sections, *Room 206, H & F Bldg.*, E. C. SCHEIDENHELM, *Presiding*.

A. Report of Dairy Cattle Breeding Committee—E. J. PERRY, *Rutgers University*.

B. Report of Breeds Relations Committee—H. A. HERMAN, *University of Missouri*.

C. Report of Pasture and Roughage Committee—R. B. BECKER, *University of Florida*.

#### Afternoon Session

1:00- 4:00 **General Session**—Post War Problems of Dairying. (See General Program.)

4:00- 5:00 **Committee Meetings.**

#### Thursday, June 22

##### Morning Session

9:00-11:00 **Joint Symposium**—Production and Extension Sections, *Room 206, H & F Bldg.*

**Mastitis and Feed Situation**—DWIGHT ESPE, *Presiding*.

See General Program.

11:00-12:00 **Business Meeting**—Extension Section.

##### Afternoon Session

1:00- 3:30 **General Session**—Latin American Dairying. (See General Program.)

3:30 **General Business Meeting**, *Room 200, Campbell Hall*.

## Evening

7:00

Annual Banquet—Installation of Officers, Presentation of Borden Awards.

## PRODUCTION SECTION

*Tuesday, June 20*

Afternoon Sessions

DWIGHT ESPE—*Chairman*

## 1:00– 4:00 Sectional Programs.

SECTION A—Feeds and Feeding, *Room 100, B & Z Bldg.*

G. W. SALISBURY, *Presiding*

- P1. Some Factors Affecting the Nutritive Value of Korean Lespedeza Hay—H. A. HERMAN, E. W. SWANSON, AND A. C. RAGSDALE, *University of Missouri.*
- P2. The Feeding Value of Cheat for Dairy Cows—M. H. BERRY, K. L. TURK, AND L. A. MOORE, *University of Maryland.*
- P3. Silage Fermentation Losses—A. E. PERKINS, *Ohio Agricultural Experiment Station.*
- P4. Silage Versus Winter Pasture for Dairy Cows—C. E. WYLIE, B. P. HAZELWOOD, AND L. R. NEEL, *University of Tennessee.*
- P5. Effect of Feeding Corn and Alfalfa Silage on the Fat- and Water-Soluble Vitamins—J. J. STEFANIAK, I. W. RUPEL, AND W. H. PETERSON, *University of Wisconsin.*
- P6. New Feeding Standard for Milk Production—T. A. BAKER, *University of Delaware.*
- P7. Limited Grain Ration Versus All Roughage Ration for Dairy Cattle—F. B. WOLBERG, A. A. SPIELMAN, V. L. MILLER, AND U. S. ASHWORTH, *State College of Washington.*
- P8. Limited Grain Feeding of Dairy Cows—C. E. WYLIE, S. A. HINTON, AND B. P. HAZELWOOD, *University of Tennessee.*
- P9. Carotene Losses in Freshly Cut Plant Tissues—R. K. WAUGH, S. M. HAUGE, AND J. H. HILTON, *Purdue University.*
- P10. Vitamin A Requirements of Dairy Cattle for Normal Growth and Reproduction—J. H. HILTON, J. W. WILBUR, AND S. M. HAUGE, *Purdue University.*
- P11. Carotene Levels for Growth and Reproduction in Dairy Bulls—I. R. JONES, *Oregon State College.*



- P12. Changes in Blood-plasma Carotene Associated with Parturition and Lactation of Jersey Cows—A. H. KUHLMAN AND W. D. GALLUP, *Oklahoma A & M College*.
- P13. Vitamin E in the Nutrition of Cattle—T. W. GULLICKSON, L. S. PALMER, W. L. BOYD, AND F. C. OLSON, *University of Minnesota*.

*Paper to be Read by Title*

- P13a. The Value of Fat in Alfalfa Hay Rations—I. R. JONES, *Oregon State College*.

SECTION B—Physiology and Nutrition, Room 209, B & Z Bldg., DWIGHT ESPE, *Presiding*

- P14. Studies on Ketosis in Dairy Cattle—J. C. SHAW, *University of Connecticut*.
- P15. Symptoms of Scurvy Observed in a Herd of Dairy Cattle—C. W. DUNCAN, C. F. HUFFMAN, R. MITCHELL, JR., AND J. T. REID, *Michigan State College*.
- P16. The Relation of Vacuum to Speed in Mechanical Milking—VEARL SMITH AND W. E. PETERSEN, *University of Minnesota*.
- P17. Studies of Mammary Gland Carbohydrate Metabolism in Vitro—C. B. KNOTT AND W. E. PETERSEN, *University of Minnesota*.
- P18. Pre-partum Milking—E. A. KEYES, J. J. REID, A. A. BORLAND, A. L. BEAM, AND P. S. WILLIAMS, *Pennsylvania State College*.
- P19. The Use of Simplified Diets in the Study of the Fat Metabolism of the Mammary Gland—O. W. KAUFMANN, *University of Connecticut*.
- P20. The Effect of Two Different Methods of Feeding Cod Liver Oil on Fat Test in Milk—L. A. MOORE, G. T. HOFFMAN, AND M. H. BERRY, *University of Maryland*.
- P21. Further Observations on the Initiation and Maintenance of Lactation in Dairy Cattle—RALPH P. REECE, *New Jersey Agricultural Experiment Station*.
- P22. The Response of Louisiana Milk Cows to Iodinated Casein Feeding—D. M. SEATH, CECIL BRANTON, AND A. H. GROTH, *Louisiana State University*.
- P23. Studies in the Utilization of Thyroprotein by Ruminants—C. W. TURNER AND E. P. REINEKE, *University of Missouri*.

- P24. Studies of Thyroid Physiology by Use of Thiourea and Its Derivatives—E. P. REINEKE, A. B. SCHULTZE, AND C. W. TURNER, *University of Missouri*.
- P25. The Effect of Pitocin on Milk Lipase—PHILIP L. KELLY, *University of Arkansas*.
- P26. Factors Affecting the Chylomicron Count—DWIGHT ESPE AND C. Y. CANNON, *Iowa State College*.

*Paper to be Read by Title*

- P27. Morphology of the Teat in Relation to Milking and Trauma—W. E. PETERSEN, C. B. KNOTT, AND W. L. BOYD, *University of Minnesota*.

4:00–5:00 Committee Meetings.

7:00 Symposium—Relation of Body Size to Milk Yield. (See General Program.)

*Wednesday, June 21*

*Morning Session*

9:00–11:00 Sectional Programs.

SECTION A—Feeds and Feeding—Calf Rearing—Harvesting Roughages, *Room 100, B & Z Bldg., DWIGHT ESPE, Presiding*

- P28. Threshed Peanut Hay as a Roughage for Dairy Cows—A. H. KUHLMAN AND H. W. CAVE, *Oklahoma A & M College*.
- P29. The Use of Urea in Commercial Dairy Feeds—W. H. HASTINGS, *Nutritional Director, Lindsey-Robinson & Company*.
- P30. Corn Silage Made with the Addition of Urea and Its Feeding Value—T. E. WOODWARD, *Bureau of Dairy Industry*.
- P31. Urea Sorghum Silage—GEORGE K. DAVIS, C. L. COMAR, R. B. BECKER, AND P. T. DIX ARNOLD, *University of Florida*.
- P32. Urea Treated Corn Silage Versus Untreated Corn Silage as a Dairy Feed—G. H. WISE, J. H. MITCHELL, J. P. LAMASTER, AND D. B. RODERICK, *Clemson Agricultural College*.
- P33. The Minimum Protein Requirement of Young Holstein Calves—L. E. HARRIS AND J. K. LOOSLI, *Cornell University*.
- P34. Studies on Carotene Conversion in the Young Calf—NORMAN S. LUNDQUIST AND PAUL H. PHILLIPS, *University of Wisconsin*.

- P35. Observations on the Pathology of Dairy Calves on Low Vitamin A Diets—E. A. KEYES, S. I. BECHDEL, W. T. S. THORP, AND N. B. GUERRANT, *Pennsylvania State College*.
- P36. The Effect of Sulfa Drugs and Immunity Development Upon Blood Plasma Vitamins A and C in the Young Bovine—NORMAN S. LUNDQUIST AND PAUL H. PHILLIPS, *University of Wisconsin*.
- P37. The Use of Stored Colostrum to Replace Marketable Milk for Calf Feeding—N. N. ALLEN, *University of Vermont*.
- P38. Experiences with the Forage Crop Harvester in Making Grass Silage—C. F. MONROE, *Ohio Agricultural Experiment Station*.
- P39. Development of the Barn Hay-drying System—C. E. WYLIE AND JOHN A. SCHALLER, *University of Tennessee*.

SECTION B—Animal Breeding and Reproduction, Room 209, B & Z Bldg., G. W. SALISBURY, *Presiding*

- P40. Congenital Muscular Contracture and Ankylosis in Dairy Cattle—A. A. SPIELMAN, O. J. HILL, AND E. C. McCULLOCK, *State College of Washington*.
- P41. The Optimum Emphasis on Dam's Records when Proving Dairy Sires—JAY L. LUSH, *Iowa State College*.
- P42. Tendency Toward Mastitis in Two Cow Families—J. M. MURPHY AND K. O. PFAU, *New Jersey Agricultural Experiment Station*.
- P43. The Effect of the Oral Administration of Chloretone to Bulls on the Fertility of the Semen Produced—IRVINE ELLIOTT, *Cornell University*.
- P44. Storage of Bovine Spermatozoa in Diluents Containing Certain Amino Acids—C. E. KNOOP, *Ohio Agricultural Experiment Station*.
- P45. Recent Observations on the Preservation of Bull Semen—HENRY A. LARDY AND PAUL H. PHILLIPS, *University of Wisconsin*.
- P46. Further Studies of the Effect of Dilution Rate on the Fertility of Bull Semen Used for Artificial Insemination—G. W. SALISBURY, IRVINE ELLIOTT, AND N. L. VANDEMARK, *Cornell University*.
- P47. Conception Rate in Dairy Cattle by Artificial Insemination at Various Intervals Before and After

Ovulation—G. W. TRIMBERGER AND H. P. DAVIS, *University of Nebraska*.

P48. The Methylene Blue Reduction Test and Its Relation to Other Measures of Quality in Bull Semen—G. W. SALISBURY, ERNEST MERCIER, AND N. L. VANDEMARK, *Cornell University*.

P49. The Influence of Pregnancy on the Body Weight of Dairy Cows—D. N. PUTNAM AND H. O. HENDERSON, *University of West Virginia*.

P50. Factors to Consider in Long Distance Semen Shipping—H. A. HERMAN AND E. W. SWANSON, *University of Missouri*.

11:00–12:00 **Joint Business Meeting**—Production and Extension Sections, *Room 206, H & F Bldg.*, E. C. SCHEIDENHELM, *Presiding*.

A. Report of Dairy Cattle Breeding Committee—E. J. PERRY, *Rutgers University*.

B. Report of Breeds Relations Committee—H. A. HERMAN, *University of Missouri*.

C. Report of Pasture and Roughage Committee—R. B. BECKER, *University of Florida*.

#### Afternoon Session

1:00– 4:00 **General Session**—Post War Problems of Dairying. (See General Program.)

4:00– 5:00 **Committee Meetings.**

#### Thursday, June 22

##### Morning Session

9:00–11:00 **Joint Symposium**—Production and Extension Sections, *Room 206, H & F Bldg.*

**Mastitis and Feed Situation**—*See General Program.*

11:00–12:00 **Business Meeting**—Production Section, *Room 100, B & Z Bldg.*

##### Afternoon Session

1:00– 3:30 **General Session**—Latin American Dairying, *Room 200, Campbell Hall*.

3:30 **General Business Meeting**, *Room 200, Campbell Hall*.

##### Evening

7:00 **Annual Banquet**—Installation of Officers and Presentation of Borden Awards.

## MANUFACTURING SECTION

*Tuesday, June 20*

Afternoon Session

P. F. SHARP—*Chairman*

## 1:00—4:00 Sectional Programs.

SECTION A—Bacteriology, *Room 302, Campbell Hall*

- M1. Factors Which Influence the Apparent Survival of Heat-Treated Bacteria—F. E. NELSON, *Iowa State College*.
- M2. A Correlation of the Resazurin Test Read with an All-Purpose Lovibond Comparator at 10, 30, and 60 Minutes with the Standard Plate Count of Milk—S. IRENE JORGENSEN AND N. S. GOLDING, *State College of Washington*.
- M3. Mastitis and the Plate Count of Milk—E. O. ANDERSON, *University of Connecticut*.
- M4. Behavior of Bacteria in Certain Gases under Pressure—M. J. PRUCHA, *University of Illinois*.
- M5. Bactericidal Property of Some Acids, Wetting Compounds, and Acid Cleaners—M. J. PRUCHA, *University of Illinois*.
- M6. Farm Sources of *Oospora Lactis*—E. R. GARRISON, *University of Missouri*.
- M7. Further Observations on the Use of the Propionates as Inhibitors of Mold on the Surface of Butter—J. C. OLSON AND H. MACY, *University of Minnesota*.
- M8. The Mold Mycelia Count as an Index of Quality of Butter—LT. P. R. ELLIKER AND B. E. HORRALL, *Purdue University*.
- M9. An *Aerobacter* Species in Whey Cream as a Cause of a Medicinal Flavor Encountered in Butter—T. J. CLAYDEN, *University of Arkansas*.

SECTION B—Chemistry, *Room 200, Campbell Hall*

- M10. Some Chemical and Physical Properties of Washing Powder—P. S. LUCAS, *Michigan State College*.
- M11. Factors Entering into the Testing of Some Cream for Extraneous Matter—K. M. RENNER, *Texas Technological College*.
- M12. The Problem of Extraneous Matter in Cheddar Cheese—W. V. PRICE AND RAYMOND MIERSCH, *University of Wisconsin*.

- M13. Correlating Lactic Acid Determination with Practical Milk Quality Test in Grading Milk for Manufactured Dairy Products—I. A. GOULD, *Michigan State College*.
- M14. Use of Conductivity Measurements for Detecting Neutralization of Sweet Cream Buttermilk—S. T. COULTER AND R. W. KUNKEL, *University of Minnesota*.
- M15. An Improved Technique for the Determination of the Volatile Acidity of Cheese—K. L. SMILEY AND A. C. DAHLBERG, *Cornell University*.
- M16. A Study of Some of the Substances Adsorbed on the Fat Globules of Milk—ROBERT JENNESS, *University of Minnesota*.
- M17. Some Factors Affecting the Inversion of Sucrose—T. R. FREEMAN AND E. L. FOUTS, *University of Florida*.
- M18. Study of the Peroxide Value of Stored Spray-Dried Whole Milk Powder—HARRY PYENSON, P. H. TRACY, AND J. E. TRIMBLE, *University of Illinois*.
- M19. The New Method for the Determination of Lipase in Milk. PHILIP L. KELLY, *University of Arkansas*.

4:00–5:00 Committee Meetings.

Wednesday, June 21

Morning Session

P. F. SHARP—*Chairman*

9:00–11:00 Sectional Programs.

SECTION A—Butter and Cheese, Room 203,  
*Campbell Hall*

- M20. The Influence of Butter Cultures and of Butter Flavors on the Quality of Butter—H. C. OLSON AND P. E. JOHNSON, *Oklahoma A and M College*.
- M21. Effect of Various Bacteria on Diacetyl Content and Flavor of Butter—LT. P. R. ELLIKER, *Purdue University*.
- M22. The Effect of Storage Temperatures on the Keeping Quality of Butter—C. W. STARK, J. R. CAMPBELL, AND E. S. GUTHRIE, *Cornell University*.
- M23. Butter Studies—E. S. GUTHRIE, *Cornell University*.
- M24. Action of Lipases from Various Sources on the Fat of Cheddar Cheese—F. J. BABEL, *Iowa State College*.

- M25. Combining Lactic and Bulgaricus Fermentation in Cheese Making to Prevent Gas Formation in Cheese Ripening—N. S. GOLDING, *State College of Washington*.
- M26. Does the Growth of Blue Mold in Roquefort Type Cheese Change Its Bacterial Flora?—N. S. GOLDING, *State College of Washington*.
- M27. Effect of Heat Treatments of Milk on Quality and Ripening of Cheddar Cheese—A. O. CALL AND W. V. PRICE, *University of Wisconsin*.
- M28. Relation of Corn and Alfalfa Silage to the Quality of Cheese and Its Carotene and Vitamin A Content—W. V. PRICE, K. HIGUCKI, AND W. H. PETERSON, *University of Wisconsin*.

SECTION B—Milk and Other Dairy Products,  
*Room 200, Campbell Hall*

- M29. Two Years' Experience in Deaerating Milk—E. S. GUTHRIE, *Cornell University*.
- M30. The Relationship of the Individuality of the Cow to the Production of Rancid Milk—W. A. KRIENKE, *Oklahoma A & M College*.
- M31. Milk as a Frozen Food—J. G. LEEDER AND F. J. DOAN, *Pennsylvania State College*.
- M32. The Utilization of Skimmilk in Ice Cream Mix—W. S. ARBUCKLE, C. N. SHEPARDSON, AND H. M. WALLING, *A & M College of Texas*.
- M33. Factors Affecting the Oxygen Content of the Gaseous Phase of Packaged Whole Milk Powder—JOHN HETRICK AND P. H. TRACY, *University of Illinois*.
- M34. The Keeping Quality of Commercial Dried Whole Milks Packaged in Air and in Nitrogen—G. R. GREENBANK, P. A. WRIGHT, AND E. F. DEYSHER, *Bureau of Dairy Industry, U.S.D.A.*
- M35. Further Observations Dealing with the Behavior of Ascorbic Acid in Evaporated Milk—D. V. JOSEPHSON AND F. J. DOAN, *Pennsylvania State College*.
- M36. A Comparison of Different Types of Sweetening Agents in the Preservation of Condensed Milk—W. A. HOSKISSON AND P. H. TRACY, *University of Illinois*.
- M37. Iron Content of Evaporated Milk in Relation to Greenish-Dark Discoloration in Mixtures of Coffee

and Evaporated Milk—W. C. COLE AND N. P. TARASSUK, *University of California*.

M38. Can We Hold Our Wartime Marketing Gains in Post-War Adjustments—C. G. McBRIDE, *Ohio State University*.

11:00–12:00 **Business Meeting**—Manufacturing Section, *Room 200, Campbell Hall*.

Afternoon Session

1:00– 4:00 **General Session**—Post War Problems of Dairying. (See General Program.)

4:00– 5:00 **Committee Meetings**.

*Thursday, June 22*

Morning Session

P. F. SHARP—*Chairman*

9:00–11:00 **Symposium**—Dehydrated Milk and Milk Products, *Room 200, Campbell Hall*.

See General Program.

11:00–12:00 **Business Meeting**—Manufacturing Section, *Room 200, Campbell Hall*.

Afternoon Session

1:00– 3:30 **General Session**—Latin American Dairying. (See General Program.)

3:30 **General Business Meeting**, *Room 200, Campbell Hall*.

Evening

7:00 **Annual Banquet**—Installation of Officers and Presentation of Borden Awards.



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## RED MOLD ON BLUE CHEESE<sup>1</sup>

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During recent years there has been a great increase in the blue cheese production of the United States, particularly in the Middle West. As new plants have come into production and the older plants have increased their output, various problems have become important. One of these is the appearance of red areas of mold growth on the surfaces of cheese in certain curing rooms.

Commonly the red mold is first evident on the cheese as small, bright-red colonies which may be few or very numerous. The colonies usually are noted in 10 days to several weeks and increase in size, often rather rapidly, until they are 1 inch or more in diameter. If the colonies are rather numerous they grow together and form large red areas which make up a considerable portion of the cheese surface. Both the colonies and areas of mold growth have more or less irregular edges. In some instances blue or green mold growth is evident in the red growth, especially when the latter has been present for some time, but ordinarily the red growth continues to be dominant in the areas in which it has developed. The red colonies and areas are very conspicuous on cheese because of their bright-red color which contrasts sharply with the colors of the other growth on the surfaces of the cheese.

### ISOLATION OF THE RED MOLD

Although the general appearance of the red colonies suggests that isolation of the mold would be simple, difficulties were encountered in the early attempts. Material taken from the colonies and smeared on various media usually gave such a heavy growth of bacteria, yeasts and *Penicillium roqueforti* that colonies of the red mold were not obtained. Eventually a cheese agar which had been developed for the isolation of *Bacterium linens* (1) was found to be fairly satisfactory.

To prepare the medium, 100 g. of ripened cheese is suspended in 300 ml. of distilled water containing 10 g. of potassium citrate. When the cheese is well distributed the mixture is warmed to about 50° C. and placed in a

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<sup>1</sup> Journal Paper No. 1153 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 119.

cylinder for separation of the fat. Thirty minutes are enough to give reasonably complete separation. Only the aqueous portion of the suspension is used in the agar so the fat is removed by suction. Ten g. of peptone, 50 g. of sodium chloride, 2 g. of sodium oxalate and 15 g. of agar are dissolved in 700 ml. of distilled water. The cheese suspension is added to this mixture and the reaction is adjusted to pH 7.4. The agar is dispensed in bottles and sterilized in the autoclave for 25 minutes at 15 pounds pressure. When plates are to be poured, the melted and cooled agar must be thoroughly agitated to distribute the suspended cheese solids.

Material taken from the red colonies on cheese and smeared on the surface of the cheese agar regularly showed growth of various organisms but usually some areas were found on which the red mold was growing away from other species. By picking material from such areas and smearing on another cheese agar plate and repeating this procedure, pure cultures eventually were obtained, although a considerable period was required. Incubation of the plates at 18° to 21° C. with a relatively high humidity was satisfactory.

#### IDENTITY OF THE RED MOLD

Laboratory examination of the red mold disclosed it to be a fungus which has been known as a contaminant of cheese for many years. It grows very

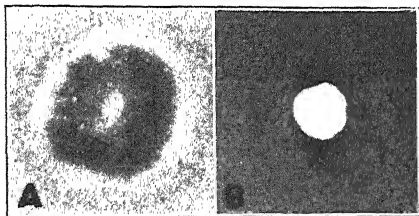


FIG. 1. Growth of *Sporendonema casei* on cheese agar (A) and potato dextrose agar (B) at 20° C., after 10 days.

slowly on the ordinary media used in mycological work, such as potato dextrose or Czapek's solution agar, but much more rapidly on the cheese medium mentioned above. This relationship explains the difficulty encountered when isolations were first attempted. On the cheese medium, the mycelium was floccose, white at first but soon becoming orange-red as the fruiting developed. On potato dextrose agar the growth was much slower, as is shown in figure 1 which illustrates colonies of the same age on the two media. On potato dextrose agar a further difference was noted; the red color was suppressed in the fungus but diffused into the medium turning it dark red. Optimum growth occurred between 15° and 20° C.

Sporulation was initiated by the production of erect fruiting hyphae on the surface of a colony. These hyphae were club-shaped and sparingly

branched, with septa at irregular intervals. They were two to three times the diameter of the mycelial threads that bore them (figure 2). Spores were laid down in the cells of the sporophores by the formation of thick walls within the walls of the sporophores. They were filled with oil drops which gave the characteristic color to the colonies. The spores were disc-shaped with rounded corners and were liberated by the breaking of the hyphal wall which surrounded them. They were variable in size depending on their position in the club-shaped fruiting hyphae; their longest diameter varied from 6 to 9 microns. These spores, being produced within the walls of the hyphae which bear them and also because of the thick wall, would be best denoted as chlamydo-spores, following the proposal of Vuillemin (17),<sup>2</sup> rather than conidia so that they would not be confused with similar thin-walled spores (arthrospores) produced on the hyphae of such fungi as

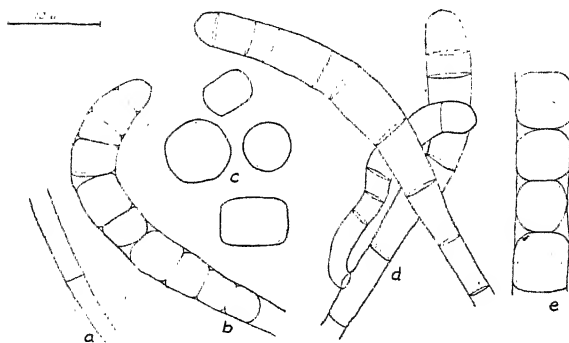


FIG. 2. Chlamydo-spores and sporophores of *Sporendonema casei*. a. Mycelium. b. Mature sporophore showing method of fracture. c. Mature conidia. d. Immature sporophores showing method of branching. e. Fragment of sporophore containing four mature chlamydo-spores.

*Geotrichum candidum* (*Oospora lactis*) as described by Langeron and Talice (8).

Taxonomically the name that should be used for the red mold is very difficult to determine. In 1826, Desmazières (6) described a red mold on cheese under the name of *Sporendonema casei* which he pointed out had been earlier denoted *Mucor crustaceus* Bulliard, *Aegerita crustacea* DeCandolle, *Oidium rubens* Link, and *Scpedonium caseorum* Link, but which did not fit into any of these genera because of the endogenous production of the spores, a character which had not been observed by earlier workers. Corda (5), in

<sup>2</sup> In 1910, Vuillemin (17) divided the asexual spore forms found in the Fungi Imperfecti into two main groups (a) *conidia vera*, i.e., spores abstricted from the tip of a conidiophore and (b) *thallospores* which are part of the hypha bearing them. The thallospores were in turn divided into three types (a) *arthrospores* which arise by fragmentation of the hypha, (b) *blastospores* which arise as globular buds and (c) *chlamydo-spores*, thick-walled spores, laid down either terminally or intercalarily.

1838, denied the internal origin of the spores and transferred the fungus to his genus *Torula* and Saccardo (14), in 1880, emended the genus *Oospora* and, in 1882, included the red mold in it under the name *Oospora crustacea* (Bull.) Sacc. Since then this name has often been used for the organism. However, in 1924, Loubière (11) in his study of the molds found on cheese returned to *Sporendonema casei* as a designation for the red fungus. In the meantime the fungus and its name had been variously interpreted because of the lack of uniformity of usage among workers in this group of fungi. Berkhout (2), in her discussion of the molds in the group, treated it in a literature review under the name *Oospora crustacea*. Sumstine (15) stated: "The genus *Sporendonema* was first described by Desmazières with one species, *S. casei*. This is generally considered congeneric with the so-called *Oospora lactis* (Fres.) Sacc. and synonymous with *Oospora crustacea* (Bull.) Sacc." Unfortunately *Sporendonema casei* was not available to Sumstine for, had it been, he doubtless would have recognized the characters which remove it from *Oospora* or *Oosporoidea*, which latter genus he erected to receive *Oospora lactis*, and treated it in its proper place. Biourge (3) discussed the species in his monograph on the genus *Penicillium* among the species of the section *Anomala* but did not make a change of name. Thom (16) said of this culture: "There is no suggestion of relationship to *Penicillium* in this culture." Still more recently Linder (10), in his revision of the genus *Oidium*, suggested that his proposed usage of the name *Oidium* might clear the way "for accepting *Sporendonema* of Desmazière for those forms represented by *Oidium* or *Oospora lactis* and *casei*," thus continuing to include *O. lactis* in the same genus with the cheese mold. In the meantime the name *Sporendonema* had been applied by Oudemans (12), in 1886, to another species, *S. terrestre*, by an emendation of Desmazières' description, a change accepted by Saccardo (14) and Lindau (9). Still later, in 1934, Ciferri and Redaelli (4) and Redaelli and Ciferri (13) again emended the genus to include certain fungi that were human pathogens. Dodge (7, p. 198) stated of Oudemans' fungus: "It does not seem related to Desmazières' original species" and that Ciferri and Redaelli "have used the name for wholly unrelated organisms."

The question of the name for the red cheese mold, then, becomes a question of the propriety of using *Sporendonema* or *Oospora* for this fungus, as well as one of the specific name. *Oospora* as a genus seems questionable (8) and certainly has not been considered to have endogenous spores, such as are found in the cheese mold. Further, the spores in that fungus are thick-walled chlamydospores rather than the thin-walled arthrospores characteristic of *O. lactis*, a species that has been suggested as congeneric. Hence Desmazières' name *Sporendonema* would seem to be the name to retain together with his specific name *casei* which was recognized by Fries. Such a procedure would mean a return to the Desmazières' genus and a discarding

of the emendations thereof, and would clearly separate *Sporendonema casei* from *Oospora lactis*, a fungus that is not congeneric.

#### CONTROL OF THE RED MOLD

In some curing rooms in which the red mold is very conspicuous on the cheese surfaces, it appears to be difficult to control; at least, the plant operators believe this is the case and certainly the mold quickly develops on young cheese going into the rooms. In other curing rooms in which the red mold has been noted, but in which it has never been conspicuous, the mold appears to have been controlled by adequate cleaning of the cheese surfaces through scraping or washing. A factor that probably is of importance in this general connection is the period the cheese is in a curing room; with short curing there apparently is less chance for the mold to become thoroughly established in a room than with long curing.

A number of cheese were dipped in, or painted with, heated petrolatum to which calcium propionate and propionic acid had been added, the cheese first being dried as completely as possible by exposing them at room temperature to an electric fan. The mold inhibitors were used in different amounts and the petrolatum, which was said to melt at 55° to 58° C., was heated to 71°, 88° or 93° C. Considerable difficulty was encountered in getting a thin and uniform layer of petrolatum on the cheese and even at curing room temperatures the petrolatum remained soft and slipped when the cheese were handled.

The petrolatum tended to limit the surface growth on the cheese and most of the conspicuous growth was very near the punch holes which were made after the treatment with petrolatum. In general, it appeared that the petrolatum offered some interesting possibilities in preventing growth of the red mold and other organisms on cheese. However, a type of material which would be firmer than the petrolatum at curing room temperatures probably would be better.

#### SUMMARY

The red mold which develops on the surface of blue cheese in certain curing rooms was most easily isolated on a special cheese agar. Apparently the logical designation for the mold is *Sporendonema casei*.

Covering blue cheese with petrolatum containing mold inhibitors offers some interesting possibilities in preventing growth of the red mold on the cheese when the normal cleaning of the cheese does not.

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# OBSERVATIONS ON THE USE OF ROLLER PROCESS SWEET CREAM BUTTERMILK POWDER IN ICE CREAM\*

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## INTRODUCTION

In recent years, the ice cream industry has used limited quantities of condensed and powdered sweet cream buttermilk as sources of milk solids-not-fat for ice cream. Whitaker (12), in 1936, reviewed the status of the use of sweet cream buttermilk in ice cream and considered the interest in this product as not being very great at that time. He noted that the ice cream manufacturer became interested in the use of the product when the price of skimmilk solids was high, or, in some localities, where the supply of sweet cream buttermilk was large. The demand for milk solids has been increasing at such a rapid pace that today the by-products of the dairy industry are being utilized as sources of human food to a much greater extent than ever before. In past years, the greater bulk of creamery buttermilk has been utilized as animal feed. Attempts are being made, however, to convert more of this by-product into channels of human consumption, and there has been a marked increase in the supply of this product in response to the sharp rise in demand for all forms of milk solids-not-fat. In those localities where concentrated sweet cream buttermilk products are available, many ice cream manufacturers are turning to these products as supplementary sources of milk solids.

## REVIEW OF LITERATURE

Combs (3) was the first to report on the use of powdered sweet cream buttermilk in ice cream. From the results he obtained, using consumer preference tests, it appeared that ice cream which contained sweet cream buttermilk powder was considered equal, or slightly superior, to that which contained skimmilk powder as the source of milk solids-not-fat.

The use of sweet cream buttermilk has been found by some investigators to improve the whipping ability of ice cream mixes containing butter as the source of butterfat. Other investigators have reported less favorable results. Caulfield and Martin (2) concluded from their studies that there was no practical value in using powdered buttermilk as a means of improving the whipping ability of ice cream mixes; however, they observed a slight

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improvement. Tracy and Ruehe (9) concluded that no improvement in whipping ability resulted when buttermilk was used with butter in ice cream mixes. Whitaker (11) reported a saving of four minutes in the time required to reach the desired overrun when using buttermilk in a butter mix. The maximum overrun obtainable was also markedly increased over that of a butter and skimmilk control. He also noted that the buttermilk mixes showed less fat clumping than the control, and this phenomenon was accompanied with a lower basic viscosity. Whitaker believed that his findings suggested the presence of a material capable of stabilizing the fat emulsion in the mixes containing buttermilk and stated that the material may be lecithin. Walts and Dahle (10) conducted an experiment which paralleled that of Whitaker. They found an average saving of two minutes in whipping time when buttermilk was used along with butter in ice cream mixes, as compared with similar mixes made with butter and skimmilk. They believed that the failure of butter mixes to whip as readily as cream mixes may be explained as due to the lack of the lecithin-protein complex and that the presence of this complex in relatively large amounts in the buttermilk may have been responsible for the results they obtained. Whitaker (12), in a discussion of the advantages of using buttermilk in ice cream called attention to its higher butterfat content in comparison with skimmilk, its superior emulsifying properties, and its ability to impart a rich creamy flavor.

#### EXPERIMENTAL METHODS

The data included in this report were obtained from studies dealing with the use of roller process sweet cream buttermilk powder as the additional source of milk solids-not-fat for ice cream. The experiments were conducted on a comparative basis and roller process skimmilk powder was used as the additional source of milk solids-not-fat for all control ice creams.

*Method of obtaining the buttermilk and skimmilk products.* Arrangements were made with a nearby Twin City plant to secure skimmilk and raw sweet cream which had been obtained from the separation of a single vat of mixed whole milk of high quality. The skimmilk was flash pasteurized at about 165° F. prior to its shipment. The cream was standardized to the desired butterfat content with a portion of the skimmilk from the same separation and was then pasteurized at 165° F. for 30 minutes, cooled to 40° F., and held overnight at this temperature before churning. A standard churning procedure was employed, and the buttermilk was recovered as soon as the butter granules were of proper size.

The skimmilk and sweet cream buttermilk were dried on a "Buflovak" atmospheric drum drier at the University of Minnesota creamery. In each trial, the fluid products were preheated to 155° F. before drying and were not precondensed. The operation of the drier was standardized before collecting the powder samples by first drying at least 200 pounds of the



fluid skimmilk. After collecting the samples of powdered skimmilk, the buttermilk was dried immediately without interrupting the operation of the drier in order that both products could be obtained under similar operating conditions. All powders were analyzed for moisture, butterfat, and titratable acidity before use in ice cream mixes.

*Method of processing and freezing the ice cream mixes.* Eighty-five-pound mixes were prepared which had the following composition: 12.0 per cent butterfat, 11.0 per cent milk solids-not-fat, 15.0 per cent cane sugar, 0.30 to 0.35 per cent 225 Bloom gelatin, and 38.30 to 38.35 per cent total solids. All mixes were pasteurized at 160° F. for 30 minutes. The mixes were homogenized at the pasteurization temperature in a two-stage Colony homogenizer at pressures of 2,500 pounds and 500 pounds on the first and second stages, respectively. They were conducted directly from the homogenizer to a direct-expansion surface cooler and cooled to between 36° and 40° F. They were then aged at a temperature of 40° F. for a period of from 18 to 24 hours before freezing.

All mixes were frozen in a Creamery Package direct-expansion, batch freezer which had a capacity of 40 quarts. The batches were weighed accurately before being placed in the freezer and equal portions were prepared for freezing. Pure vanilla extract was added at the rate of 4 ounces per 40-pound batch.

Extreme care was taken to secure uniform freezing conditions when freezing a series of mixes in order that comparative freezing and whipping data could be obtained. A preliminary batch of mix was always frozen to chill the freezer and to standardize operating conditions. The time of freezing was standardized and was held constant for each series of mixes. All samples of ice cream used for meltdown tests and scoring were taken at an overrun of 90 per cent and placed immediately in a hardening room maintained at about -10° F.

*Methods of testing the ice mixes and ice cream.* The total solids content was determined on all ice cream mixes according to the method outlined by Mojonnier and Troy (5).

The titratable acidity was determined on duplicate samples of each ice cream mix within 18 hours after processing. A 9-gram sample of undiluted mix was titrated with N/10 sodium hydroxide using phenolphthalein as the indicator. The acidity was calculated as per cent lactic acid.

The pH of the mixes was determined with a Coleman Model 3 Electrometer.

The original, apparent, and basic viscosities were determined on all mixes. The original viscosity was determined on a sample of the fresh mix immediately after cooling. The apparent and basic viscosities were those of the aged mix after an aging period of about 18 hours. A sample of unagitated mix was used for the apparent viscosity measurements, whereas

the basic viscosity was that of a sample of the aged mix after it had been passed through a hand emulsifier operated at the rate of 78 strokes per minute, according to the method of Penczek and Dahlberg (6). The viscosities were determined with an Improved MacMichael viscosimeter operated at 20 revolutions per minute and using a standard disc plunger suspended by a number 31 wire. The wire was calibrated with a 60 per cent sucrose solution and all readings were converted to viscosity in centipoises. All viscosity readings were taken at a temperature of 40° F., and the operations were conducted in a room maintained at about 40° F.

All overrun determinations were made at the freezer by the use of a Mojonner Overrun Tester. The tester was accurately calibrated for each batch of mix immediately before freezing.

A general indication of whipping ability was secured for all ice cream mixes by checking the time required to whip to an overrun of 90 per cent. In each case duplicate batches were frozen for an extended whipping ability test. The frozen mix was allowed to whip until the maximum overrun had been reached and the overrun and temperature of the ice cream were determined at one-minute intervals throughout the whipping process. Temperatures were determined with a sub-zero centigrade thermometer graduated in 0.1-degree divisions. The temperature in each case was estimated to the nearest 0.05 degree and all centigrade readings were converted to degrees Fahrenheit.

During one trial, Draw-Rite readings were recorded at minute intervals during the freezing and whipping of each mix and coincidentally with each overrun determination. It was believed that since Draw-Rite readings serve as an indication of the consistency of the ice cream in the freezer, it would be possible to utilize these readings in comparing the consistencies of the ice cream throughout the freezing process.

The melting properties of the ice cream were determined by allowing a pint sample of ice cream to melt at room temperature. The ice cream (a weighed sample) was placed on a wire screen which had been placed over a glass funnel, and the drippings were collected in a beaker. The weight of drainage was determined at regular intervals until melting was nearly complete.

The ice creams were scored for flavor and body and texture by two experienced judges of dairy products. The age of the sample at each scoring was recorded along with the scores and criticisms.

## RESULTS

Representative data from three experiments are included in this report. For purposes of discussion these experiments will be referred to as trials A, B, and C.

*Trial A.* For trial A, a series of four mixes was designed as follows:

Mix number	Source of butterfat	Source of additional milk solids-not-fat
1 (control)	cream	R.P.* skimmilk powder
2	cream	R.P. sweet cream buttermilk powder
3 (control)	unsalted butter	R.P. skimmilk powder
4	unsalted butter	R.P. sweet cream buttermilk powder

\*Roller process.

The skimmilk and buttermilk powders were derived from the same lot of whole milk and supplied in each case about 46 per cent of the milk solids-

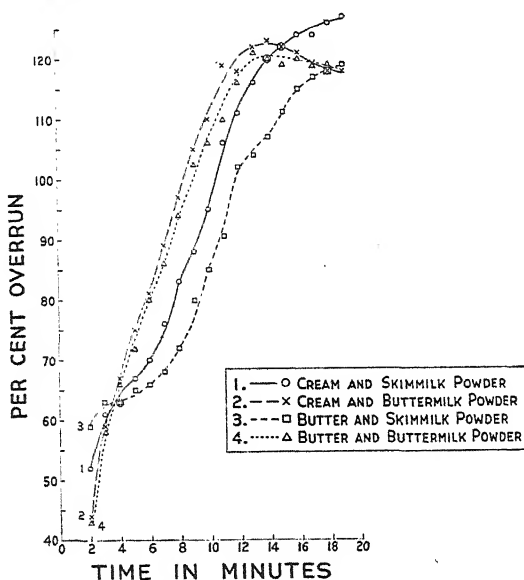


FIG. 1. The effect of roller process sweet cream buttermilk powder on the whipping ability of ice cream mixes containing cream and butter as sources of butterfat.

not-fat in the mix. Duplicate batches of each mix were frozen, and one batch was drawn at an overrun of 90 per cent, whereas the other batch was allowed to whip to the maximum overrun in order to obtain whipping ability data. The data obtained from the trial are presented in table 1 and figure 1. There were no marked differences among the viscosities of either the fresh or aged mixes in this trial. The most marked difference was evident in the rates of whipping of the mixes in the freezer. In each comparison (table 1) the mix which contained buttermilk powder attained an overrun of 90 per cent more rapidly than the skimmilk powder control, resulting in a saving in time of from about 2 to 3 minutes. In each case, it will be noted that the ice cream which contained buttermilk powder was drawn from the freezer at a lower temperature than the control. A more complete

picture of the whipping ability of the mixes is presented in figure 1. It will be noted that there was an initial "lag" in the rate of overrun development in the early stages of whipping for each of the buttermilk mixes. The greatest maximum overrun was attained in the cream and skimmilk powder mix. In each case, the ice cream which contained buttermilk powder was firmer

TABLE 1

*Trial A.—The effect of sweet cream buttermilk powder upon certain properties of ice cream mixes and ice cream containing either cream or butter as the source of butterfat*

Properties of ice cream mix	Mix			
	1	2	3	4
	Cream and skimmilk powder	Cream and buttermilk powder	Butter and skimmilk powder	Butter and buttermilk powder
Titrateable acidity (%)	0.190	0.190	0.195	0.195
pH	6.31	6.31	6.32	6.31
Original viscosity (centipoises)	34.5	35.5	34.8	35.3
Apparent viscosity (centipoises)	179.0	144.0	147.5	155.0
Basic viscosity (centipoises)	55.3	45.3	48.5	49.5
Freezing time (min.: sec.)	2: 0	2: 0	2: 0	2: 0
Whipping time (min.: sec.)	7: 5	4: 16	6: 55	4: 51
Final overrun (%)	90.0	90.0	90.0	90.0
Temperature when drawn (° F.)	24.44	23.81	24.44	23.99
Condition when drawn	Sl. soft, wet	Very firm, dry	Sl. soft, wet	Firm, sl. wet
<i>Melting properties at 75° F.</i>				
Per cent drainage				
After 60 minutes	23.6	25.3	29.7	25.4
"    90 minutes	56.8	57.0	63.9	54.7
"    120 minutes	74.2	77.7	84.3	76.6
Appearance of meltdown	Coarse foam	Fine foam	Coarse foam	Fine foam
<i>Flavor scores and criticisms</i>				
After one week	39.0 Lacks freshness, sl. cooked	38.5 Lacks freshness	39.0 Lacks freshness, sl. cooked	38.0 Lacks freshness
After four weeks	38.0 Stale	38.0 Stale	38.0 Stale	38.0 Stale
<i>Body and texture scores and criticisms</i>				
After one week	28.0 Coarse	29.0 Sl. coarse	28.0 Coarse	29.0 Sl. coarse
After four weeks	28.0 Coarse	28.5 Coarse	28.0 Coarse	28.5 Coarse

and drier in appearance when drawn from the freezer than the control. The results of the meltdown tests indicate that there were no marked differences in the rates of drainage from the melting samples. All samples were foamy, but the foam from the samples which contained buttermilk powder was noticeably finer in structure and more stable than that of the controls. The initial flavor scores of the ice cream samples which contained butter-

milk powder were slightly less than the controls, but no differences were apparent after four weeks in storage. A cooked flavor was initially present in the samples which contained skimmilk powder, but this flavor was not detected in the other samples. The buttermilk ice creams were judged better in body and texture than the controls at both the initial and final scorings.

*Trial B.* It has been conclusively demonstrated by various investigators (1, 4, 7, 8) that as the butterfat content of the cream churned is increased there is an increase in the phospholipid content of the resulting buttermilk. Trial B was therefore designed to determine the influence of variations in the phospholipid content of sweet cream buttermilk powders upon the properties of ice cream mixes and the frozen products. Roller process sweet cream buttermilk powders were prepared from buttermilks obtained by the churning of three lots of sweet cream which contained 25, 33, and 40 per cent butterfat, respectively. The lower testing creams were obtained by standardizing portions of a single lot of 40 per cent cream with skimmilk from the same separation. A quantity of the skimmilk was dried for use in the control mixes. Four ice cream mixes were prepared from cream, skimmilk, and either skimmilk or buttermilk powder as follows:

<i>Mix number</i>	<i>Source of additional milk solids-not-fat</i>
1 (control)	R.P.* skimmilk powder
2	R.P. buttermilk powder from 25 per cent cream
3	R.P. buttermilk powder from 33 per cent cream
4	R.P. buttermilk powder from 40 per cent cream

\* Roller process.

The data obtained from one set of mixes are presented in table 2, and the results of the whipping ability tests obtained from the freezing of duplicate batches are illustrated in figures 2 and 3. As shown in the table, the original, apparent, and basic viscosities were quite uniform for the entire series of mixes. Every mix which contained sweet cream buttermilk attained the desired overrun of 90 per cent in approximately 2 minutes less time than the control. An examination of the overrun-time curves of figure 2 reveals that the mixes attained practically the same maximum overrun. An initial lag in the overrun curves of the buttermilk mixes is clearly evident. The overrun-temperature relationships for each of the mixes are illustrated in figure 3. It is evident that the overruns of the mixes which contained sweet cream buttermilk powder were attained at a lower temperature than the control throughout the major part of the whipping process. As was found true in other trials, the freshly drawn ice creams which contained buttermilk powder were firmer and drier in appearance than the ice cream which contained skimmilk powder. The melting properties, flavor scores, and body and texture scores of the ice cream samples were quite uniform. The foam from the melting samples which contained buttermilk powder was finer in structure and more stable than that of the control sample. A cooked flavor was noted in the control sample but was absent in all other samples.

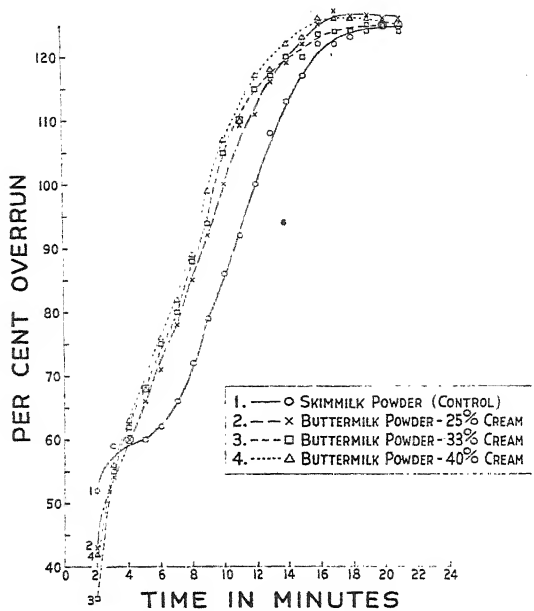


FIG. 2. The effect of roller process sweet cream buttermilk powders derived from creams of various butterfat contents on the whipping ability of ice cream mixes containing cream as the source of butterfat.

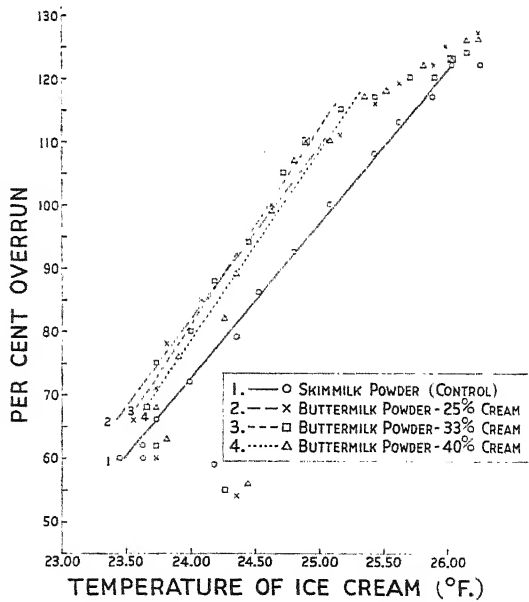


FIG. 3. The effect of roller process sweet cream buttermilk powders derived from creams of various butterfat contents on the overrun-temperature relationship of ice cream mixes.

*Trial C.* Trial C was conducted in exactly the same manner as trial B with the exception that butter was used as the source of butterfat instead of cream. The data presented in table 3 show that the results obtained practically paralleled those obtained in the previous trial. The viscosity data were more irregular, but the differences were not considered significant. It will be noted that the drainage from the melting ice cream samples which con-

TABLE 2

*Trial B.—The effect of sweet cream buttermilk powders derived from creams of various butterfat contents upon certain properties of ice cream and ice cream containing cream as the source of butterfat*

Properties of ice cream mix	Mix			
	1	2	3	4
	Skimmilk powder	Buttermilk powder*	Buttermilk powder†	Buttermilk powder‡
Titrateable acidity (%)	0.190	0.185	0.185	0.190
pH	6.42	6.41	6.42	6.42
Original viscosity (centipoises)	42.5	44.0	43.3	44.0
Apparent viscosity (centipoises)	127.5	122.0	122.3	116.5
Basic viscosity (centipoises)	44.3	47.0	45.0	45.5
Freezing time (min.: sec.)	2: 0	2: 0	2: 0	2: 0
Whipping time (min.: sec.)	7: 40	5: 55	5: 50	5: 26
Final overrun (%)	90.0	90.0	90.0	90.0
Temperature when drawn (° F.)	24.62	24.35	24.26	24.26
Condition when drawn	Firm, sl. wet	Firm, mod. dry	Firm, mod. dry	Very firm, mod. dry
<i>Melting properties at 75° F.</i>				
Per cent drainage				
After 60 minutes	19.4	20.1	21.7	19.2
" 90 minutes	49.6	50.0	51.8	47.6
" 120 minutes	69.4	71.3	73.1	71.7
Appearance of meltdown	Coarse foam	Fine foam	Fine foam	Fine foam
<i>Flavor scores and criticisms</i>				
After three days	40.0	40.0	40.0	40.0
	Cooked	.....	.....	.....
After three weeks	40.0	40.0	40.0	40.0
	Cooked	.....	.....	.....
<i>Body and texture scores and criticisms</i>				
After three days	29.0	29.0	29.0	29.0
	Sl. coarse	Sl. coarse	Sl. coarse	Sl. coarse
After three weeks	28.5	28.5	28.5	28.5
	Coarse	Coarse	Coarse	Coarse

\* Derived from 25 per cent cream.

† Derived from 35 per cent cream.

‡ Derived from 40 per cent cream.

tained buttermilk powder tended to take place at a slower rate than that of the control. The overrun curves presented in figure 4 show that the greatest maximum overrun was attained in the control mix. The differences in rate of overrun development were not as pronounced as in trial B, but otherwise the same general relationships existed. The overrun-temperature

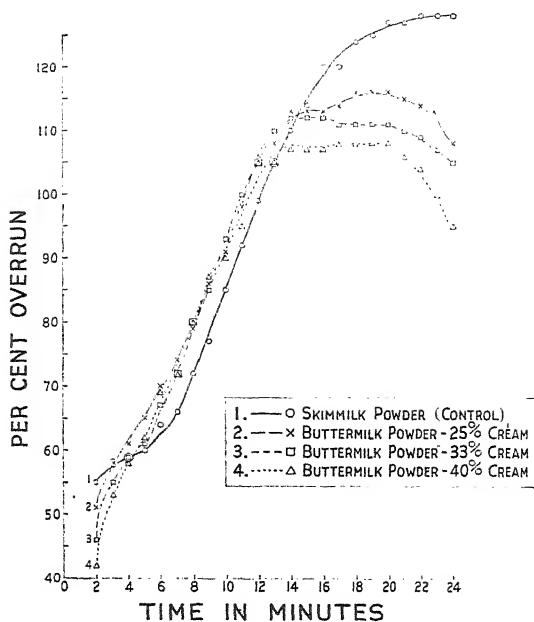


FIG. 4. The effect of roller process sweet cream buttermilk powders derived from creams of various butterfat contents on the whipping ability of ice cream mixes containing butter as the source of butterfat.

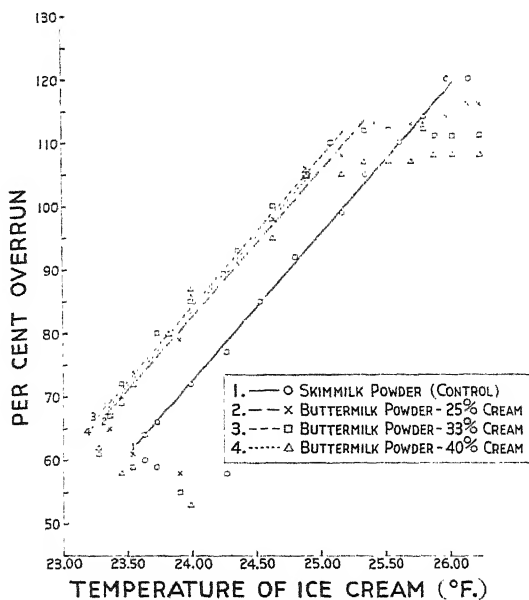


FIG. 5. The effect of roller process sweet cream buttermilk powders derived from creams of various butterfat contents on the overrun-temperature relationship of ice cream mixes containing butter as the source of butterfat.



relationships, illustrated in figure 5, show that the capacity to overrun at a given temperature was greater for the mixes which contained buttermilk powder than for the control throughout that part of the whipping process where a straight-line relationship existed. The use of Draw-Rite readings in comparing the consistencies of the ice creams throughout the freezing process served as evidence that each of the ice creams which contained sweet cream buttermilk powder was greater in consistency than the control throughout the major portion of the whipping period. The results are illustrated in figure 6.

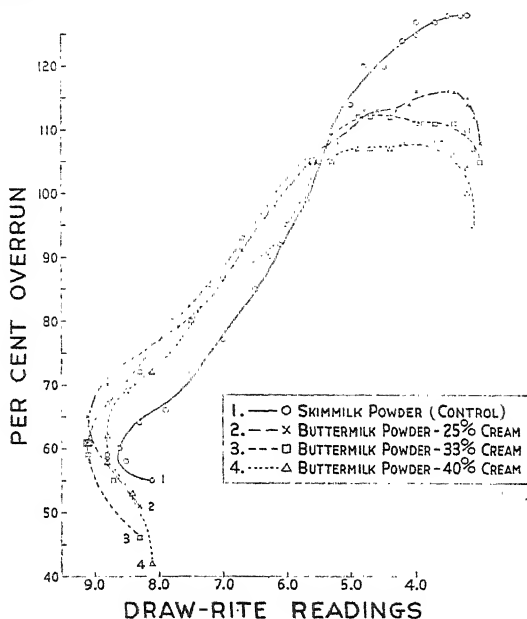


FIG. 6. The effect of roller process sweet cream buttermilk powders upon the consistency of ice cream mixes during the whipping process as measured by Draw-Rite readings.

#### SUMMARY

Experiments were conducted to determine the influence of roller process sweet cream buttermilk powder upon the properties of ice cream mixes and ice cream. The buttermilk product was used as the additional source of milk solids-not-fat for ice cream and the corresponding skimmilk product was used in the same manner in control mixes. In every comparison, the sweet cream buttermilk and skimmilk products were both derived from the same lot of mixed whole milk.

There were no consistent or marked differences in the viscosities of the ice cream mixes which could be attributed to any single factor.

TABLE 3

*Trial C.—The effect of sweet cream buttermilk powders derived from creams of various butterfat contents upon certain properties of ice cream mixes and ice cream containing butter as the source of butterfat*

Properties of ice cream mix	Mix			
	1	2	3	4
	Skimmilk powder	Buttermilk powder*	Buttermilk powder†	Buttermilk powder‡
Titratable acidity (%)	0.200	0.190	0.190	0.190
pH	6.35	6.33	6.34	6.34
Original viscosity (centipoises)	42.5	40.8	40.0	39.3
Apparent viscosity (centipoises)	133.0	160.5	118.5	105.5
Basic viscosity (centipoises)	52.0	45.0	44.0	40.5
Freezing time (min.: sec.)	2: 0	2: 0	2: 0	2: 0
Whipping time (min.: sec.)	8: 10	6: 45	6: 46	6: 0
Final overrun (%)	90.0	90.0	90.0	90.0
Temperature when drawn (° F.)	24.62	24.17	24.08	23.99
Condition when drawn	Sl. soft, wet	Mod. firm, mod. dry	Very firm, dry	Very firm, dry
<i>Melting properties at 75° F.</i>				
Per cent drainage				
After 60 minutes	43.4	34.1	32.8	38.1
“ 90 minutes	72.3	61.8	59.9	61.9
“ 120 minutes	92.4	85.5	84.2	84.6
Appearance of meltdown	Coarse foam	Fine foam	Fine foam	Fine foam
<i>Flavor scores and criticisms</i>				
After four days	39.5 Lacks freshness, cooked	39.5 Lacks freshness	39.5 Lacks freshness	39.5 Lacks freshness
After three weeks	38.5 Sl. cooked, lacks freshness	39.0 Lacks freshness	39.0 Lacks freshness	39.0 Lacks freshness
<i>Body and texture scores and criticisms</i>				
After four days	29.0 Sl. coarse	29.0 Sl. coarse	29.0 Sl. coarse	29.0 Sl. coarse
After three weeks	28.0 Coarse	28.0 Coarse	28.0 Coarse	28.0 Coarse

\* Derived from 25 per cent cream.

† Derived from 35 per cent cream.

‡ Derived from 40 per cent cream.

A consistent improvement in the whipping ability of ice cream mixes was obtained through the use of roller process sweet cream buttermilk powder as the additional source of milk solids-not-fat in place of skimmilk powder. In most trials, the desired overrun was obtained in one to two minutes less time than for the control mixes. The superior whipping ability of the mixes which contained roller process sweet cream buttermilk powder was further evidenced by the fact that the desired overrun was obtained at a lower temperature than that of the controls. Overrun-time curves and overrun-temperature curves derived from whipping tests are presented

which show that the superior whipping ability of sweet cream buttermilk mixes persisted over a wide range of overruns. The maximum overrun obtainable was not increased through the use of roller process sweet cream buttermilk powder and tended to be somewhat inhibited in butter mixes.

Ice creams which contained sweet cream buttermilk solids were observed to be drier in appearance and greater in consistency when freshly drawn from the freezer than control mixes which contained skimmilk solids.

The melting resistance of the ice creams was not markedly affected by the use of sweet cream buttermilk solids. In all comparisons, it was noted that the ice creams which contained sweet cream buttermilk solids were more foamy in appearance during melting and that the foam of the filtrate contained smaller air cells than that of the control samples.

In many comparisons, the samples of ice cream which contained sweet cream buttermilk solids were judged as richer in flavor than the controls which contained skimmilk powder. It was further noted that whereas ice cream which contained roller process skimmilk powder was often criticized for possessing a cooked flavor, this criticism was never employed in evaluating the ice creams which contained roller process sweet cream buttermilk powder.

The use of roller process sweet cream buttermilk powders derived from buttermilk obtained by the churning of sweet cream testing either 25, 33, or 40 per cent butterfat was found to influence the ice cream mixes in a similar manner. In view of these results, it appears that the normal variations in the phospholipid content of buttermilk which result from the churning of creams within the above range of butterfat contents have no significant influence upon the properties of either ice cream mixes or ice cream, the maximum effect being approached through the use of buttermilk from 25 per cent cream.

#### CONCLUSIONS

The following conclusions are believed justified after a careful analysis of all observations relative to the experimental work reported above:

1. Neither the original viscosity nor the apparent and basic viscosities of the aged mix are significantly affected when using roller process sweet cream buttermilk powder in place of roller process skimmilk powder as the source of additional milk solids-not-fat for ice cream.

2. The use of roller process sweet cream buttermilk powder in ice cream mixes results in a greater rate of whipping than when using roller process skimmilk powder. Usually, 1 to 2 minutes less time is required to obtain a normal overrun, and the overrun is attained at a lower temperature. This is true when either cream or unsalted butter is used as the source of butterfat for the mix.

3. The use of roller process sweet cream buttermilk powder in ice cream results in a freshly frozen product which is drier in appearance and greater in consistency than when the skimmilk product is used.

4. Roller process sweet cream buttermilk powder tends to impart a richer flavor to ice cream than roller process skimmilk powder.

5. There is less tendency toward a cooked flavor in ice cream containing roller process sweet cream buttermilk powder than when containing the skimmilk product.

6. Ice cream containing roller process sweet cream buttermilk powder is characterized by a foamy meltdown, the foam being finer in structure and more stable than that of ice cream containing roller process skimmilk powder.

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# THE ACTION OF THE MECHANICAL MILKER IN RELATION TO COMPLETENESS OF MILKING AND UDDER INJURY<sup>1</sup>

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Although many are convinced that mechanical milking can be as good or better than hand milking, two major objections to machine milking are raised by some. One objection is that the mechanical milker causes injury to the teats and udder and the other is a claim that many cows will not milk out completely by the machine, necessitating milking varying amounts by hand.

The question as to whether or not the mechanical milker can cause injury to the teat and udder is an important one. It is common knowledge that trauma to the teat and udder, that is readily detected, is followed by a high incidence of clinical mastitis. Evidence is accumulating to indicate that trauma to the inner linings of the teat and gland, not detectable by palpation, may be an important predisposing factor to mastitis infection. Kennedy (3) has reported that by careful daily examinations of the teats and udder he detected trauma in varying degrees before the mastitis organism could be found in the milk.

It is well known that some cows do not milk out completely to the machine, as it is ordinarily operated. Sometimes as much as 40 per cent or more of the milk in the udder is removed by hand milking after removal of the machine. It is commonly observed that incomplete milking by the machine is more common among older cows than among first-calf heifers. It is obvious that much of the advantage of machine milking is lost when it becomes necessary to remove varying amounts of milk by hand milking.

## THE PROBLEMS AND METHODS OF STUDY

Since a search of the literature failed to reveal any reports (except Petersen, 3) dealing specifically with either of these problems, work was instituted with a view of obtaining information toward their solution. Attempts were also made to compare the force exerted by the mechanical milker with that of hand milking.

To determine the action of the milking machine upon the udder a technique was developed whereby the excised mammary gland is used. The actual rate and completeness of milking by machine was ascertained on a number of cows by suspending the milking machine from a scale from which readings were taken every 20 seconds.

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The excised udder was suspended in the laboratory in approximately the normal position, by means of hooks in the median suspensory ligament and lateral facia. The gland cisterns were cannulated with glass cannula with a bore of 8 mm. or more. Skimmilk or physiological saline was then infused by gravity so as to maintain an intra-sinus pressure of approximately 50 mm. Hg. This is about the pressure developed in the cistern by the let down of milk. Skimmilk has been found more satisfactory than physiological saline as the latter will cause varying amounts of edema over a period of time.

Since the tonicity of the sphincter muscles surrounding the meatus of the teat is well maintained in the excised gland this preparation lends itself well to a study of mechanical milking. Observations have been made on the effect of such variables as pulsation rate, different vacuum levels, variations in size, shape and condition of the rubber inflations and others. Results of these observations will be presented in a later paper.

The most striking suitability of this preparation, however, was in the study of the action of the milking machine upon the teat and udder. After the gland had been filled with fluid through the inserted cannula the position of the teat cup on the teat could be observed with varying intra-sinus pressures, as could also the rate of milk withdrawal. The rate of inflow was regulated by adjustment of screw clamps on the rubber tubing connecting the skimmilk or physiological saline reservoir with the cannula in the sinus.

The action of the mechanical milker upon the inside of the teat and gland sinus was observed through incisions made in the lateral wall of the sinus or through a 2-inch tube inserted in an incision made perpendicularly over the orifice between the gland sinus and the teat. From the lateral incision the action could be felt by the insertion of a finger and actual measurements of the amount of suction developed within the teat were made by means of a vacuum gauge.

The force or "massage" action of the collapse of the rubber inflation upon the teat upon release of vacuum in the teat cup shell was also measured by the insertion of a small rubber bulb, attached to a manometer, into the teat sinus.

All of the observations reported herein were made by the use of double action mechanical milkers.

The measurements of the force applied to the teat in hand milking was accomplished both on the excised gland and on cows. In the excised gland this measurement was obtained by inserting a small rubber bulb attached to a mercury manometer into the teat sinus and different experienced milkers were used. The measurements on cows were taken with the same instrument, the rubber bulb being held in the palm of the hand so that pressure on the teat would cause compression thereof.

After a number of trials it was found that a rubber bulb of  $2\frac{1}{2}$  cc. capacity was the most satisfactory. This size held the minimum amount of mercury

needed and at the same time offered less interference to milking than larger bulbs. The bore of the manometer was such that it held 1 cc. mercury per 20 inches.

#### RESULTS

When the intra-gland sinus pressure remained constant in the excised gland the rate of milk withdrawal by the machine was constant for any given vacuum. With reduced intra-gland sinus pressures, the effect upon the rate of milk withdrawal varied with different glands. In some cases the rate of milk withdrawal began decreasing with a drop to 30 mm. Hg pressure and in others no detectable change was noted, until the intra-sinus pressure dropped to 10 mm. Hg or less. At the time that the rate of milk withdrawal began to decline the teat cups were noted to crawl upwards (C, fig. 1) with each vacuum stroke. The rates of crawling and decrease in milk withdrawal varied with different glands. In general it was noted that the rate of decline of milk withdrawal was more rapid when the crawling was also more rapid.

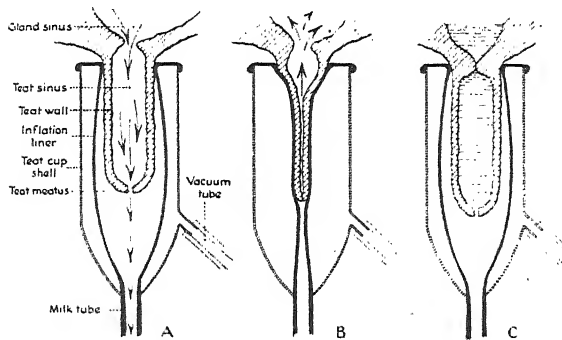


FIG. 1. Illustration of the action of the mechanical milker upon the teat. A. Vacuum applied in teat cup shell when the gland is full of milk. The teat meatus is opened and milk streams through it. B. Release of vacuum in the teat cup shell. The teat meatus is closed and a part of the milk in the teat sinus is forced back as a result of compression of the teat caused by the collapse of the inflation liner. C. Teat cup has crawled upward. The orifice between the gland and teat sinus is closed preventing passage of milk into the teat.

In 12 out of 14 cases the milk flow stopped before the gland was completely evacuated even though the machine was left attached. In all cases the gland was completely evacuated when the teat cups were pulled down part way on the teat. Once the teat cups had been permitted to crawl upward to a point where milk withdrawal had stopped it was necessary to increase the intra-gland sinus pressure by inflow of fluid to a point greater than that which would prevent the teat cup from crawling before milk withdrawal would again begin without a downward pull on the teat cups. While these values varied for different glands representative values lie between 10 and 20 mm. Hg greater pressure required to resume milk flow than is necessary to prevent the teat cups from crawling.

The reason for the teat cups crawling and the ultimate complete stoppage of milk withdrawal when the intra-sinus pressure is sufficiently reduced is seen through an incision through the sinus wall. By inserting the finger it was possible to ascertain that when the intra-gland sinus pressures were sufficiently high the orifice between this sinus and that of the teat remained wide open at all times as illustrated in A and B in figure 1. Under these conditions there is no restriction to the milk flow downward through the teat meatus upon the application of vacuum. It could also be felt that upon release from vacuum a small but definite spurt of milk flowed from the teat into the gland sinus as the result of compression of the rubber inflation upon the teat (B, fig. 1).

When the teat cups began to crawl upward as the result of reduced intra-gland sinus pressures the orifice could be felt to close. In most cases even though the gland sinus was filled with milk the closure was complete. In five instances free incisions were made so that the gland sinus communicated with the atmosphere without any air being drawn into the teat. If after the orifice between the gland and teat sinus is closed the teat cup is lowered part way down on the teat it was again opened.

Through the tube inserted perpendicularly through the gland the action of the pulsations on the tissues could be observed. It was noted that with each vacuum stroke the soft tissues surrounding the juncture of the gland and teat sinus were not only brought forcibly together but were also vigorously forced downward.

No detectable vacuum is developed in the teat as the result of milking machine action until there is partial closure of the orifice between the gland sinus and the teat. When the orifice is completely closed the vacuum within the teat sinus is the same as that in the milk line. With each release of vacuum in the shell of the teat cups the vacuum disappears within the teat because of the collapse of the rubber inflation compressing the teat.

It is difficult to obtain accurate measurements of the force exerted upon the teat by the collapse of the rubber inflation caused by the release from vacuum in the teat cup shell. The hammer-like force of the collapse has a tendency to cause the mercury, in the case of the mercury manometer, or the needle in the case of an air pressure gauge to overshoot. The values as observed are therefore only approximate. That there is a definite massaging action is certain. The approximate force is from 25 to 60 mm. Hg. Variations in the amount of force exerted were observed between different inflations of the same type, the reasons for which are obscure.

In a subsequent report factors affecting the speed of milking will be dealt with in more detail. Here will be presented only those observations on milking by machine that contribute directly to the action of the machine. In figure 2 is presented the results of milking a cow, first with the machine left on until no more milk was obtained, then followed by hand milking and



secondly, when the teat cups were drawn part way down at the time the rate of withdrawal began to decrease. It will be noted that the rate of milk withdrawal was constant for the first 2 minutes after which it decreased rapidly, with no manipulation of the machine, stopping completely in about  $4\frac{1}{2}$  minutes. After removal of the machine 2.1 pounds of milk were obtained by hand milking. When the teat cups were pulled partly down on the teats the rate of withdrawal did not begin to decrease until after 2 minutes, 40 seconds, and the gland was completely emptied without hand milking in 3 minutes.

Without going into detail it must be stated that great variation exists between different cows in the type of curve presented by plotting rates of

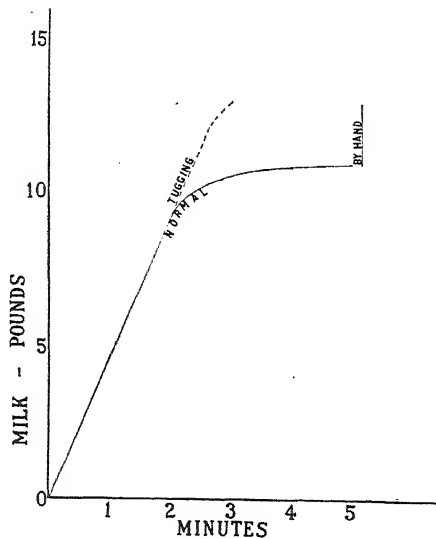


FIG. 2. Illustration of the effect of tugging upon the teat cups, when they begin to crawl, upon the rate and completeness of milking.

milk withdrawal by machine. So far, however, no cow in the University herd cannot be completely milked out by machine. For the majority of cows it is necessary to draw the teat cups downward to effect a complete evacuation of all of the milk in the gland.

The attempts at determining the force exerted upon the teat in hand milking was found to be difficult and not too satisfactory. The presence of the rubber bulb either in the teat sinus, in the excised gland, or in the palm of the hand in natural milking undoubtedly contributed to changes in the force applied. The inertia of the mercury is another factor contributing to error in the results that must not be overlooked. The values observed, therefore, must not be taken as absolute but merely indicative of the force exerted.

Measurements taken on four milkers varied from 16 inches to 24 inches of Hg pressure on the same cow. One fast milker exerted as much as 32 inches of Hg pressure in milking a hard-milking cow.

#### DISCUSSION OF RESULTS

The observations on the action of the mechanical milker upon the teat and udder in relation to varying intra-gland sinus pressures explains the observed curve characteristics when the rate of milk withdrawal is plotted. It is also in accordance with what would be expected on the basis of known physical laws. As all of the observations reported herein were made on the double action mechanical milker there is a continuous vacuum on the milk tube and alternate vacuum and release in the teat cup shell. The application of vacuum in the teat cup shell draws the rubber inflations toward the teat cup walls, permitting the full force of the continuous vacuum in the milk line to act upon the teat. Since this force is exerted equally in all directions, there is a tendency for the teat to be pulled down into the teat cup. This pulling down of the teat and lower part of the udder is inhibited by two other factors—the rigidity of the tissues as a result of the pressure of the milk within the gland and a partial displacement of the void by the outrushing milk through the meatus.

As milk withdrawal progresses the intra-glandular pressure is reduced and the tissues offer less resistance to the sucking action and are thus drawn farther into the teat cup. As the tissues at the juncture of the teat and gland sinuses are drawn into the teat cup the orifice between the two becomes occluded and further flow of milk from the gland is first decreased and finally completely stopped. When this occurs it is obvious that the full force of the vacuum is applied to the drawing in of the teat and lower part of the udder. Since the teat meatus is opened it is to be expected, as was found, that the vacuum within the teat is identical with that in the milk line.

In comparing the force of mechanical and hand milking it is significant that normal good hand milkers exert a greater force upon the teat than does mechanical milking with the usual vacuum. Also that the force exerted in hand milking is continuous throughout the entire milking process while in mechanical milking there is no measurable force exerted on the inside of the teat until the rate of milk withdrawal is reduced or completely stopped. It is possible that mechanical milking may have a greater traumatizing effect because of a somewhat shearing action than the straight compression action of hand milking. However, it is possible to so manage milking by machine, by removing it when free flow of milk ceases, that less injurious action will result to the teat and udder than with hand milking. On the other hand, if the machine is left on for prolonged periods after milk ceases to flow it can be injurious.

A well-established but little-recognized anatomical fact is the location of accessory secreting glands (4) in the gland sinus and in the upper part of

the teat sinus. These accessory glands have but a single layer of columnar epithelial cells as contrasted to the two layers of pavement epithelium lining the major portion of the teat sinus and are therefore more easily injured. The greatest compression in hand milking, particularly, in stripping and the most vigorous action of the milking machine is on this vulnerable area.

Upon the release from vacuum the rubber inflation collapses to mildly compress the teat and thus help prevent congestion. Before the teat is compressed the meatus closes leaving the teat sinus full of milk. Any further compression on the teat causes milk to be forced upward into the gland sinus. Since the compression of the teat is not complete not all of the milk in the teat sinus is forced back.

Variations in the point at which the teat cups begin crawling and in the amounts of milk left in the gland after milk withdrawal (without force applied to the teat cups) can tentatively be explained on an anatomical basis. Where the anatomical structure is such as to offer greater resistance to being drawn into the teat cup it is obvious that milking will be more complete before there is occlusion between the teat and gland sinuses. Great differences have been observed in the extent of the annular ring at the juncture of the teat with the gland sinus. In some cases this annular ring may extend more than half way across the orifice from one side to conceivably help cause an earlier occlusion during milking.

The observation that once the teat cups have crawled upwards to occlude passage from the gland sinus to the teat considerable increase in intra-gland sinus pressure is needed to again resume milk withdrawal explains in part why more rapid milking is obtained when the cow has been stimulated to let down milk before milking. Another factor is that unless let down has taken place the gland sinuses are emptied and the machine is acting for varying lengths of time without removing any milk before they are again filled. Ely and Petersen (1) reported that about 45 seconds intervened between the secretion of the hormone responsible for the let down of milk and the actual let down. In practice, therefore, it is suggested that the stimulation for the let down should take place about one minute before attaching the mechanical milker.

#### SUMMARY AND CONCLUSIONS

1. A technique is described whereby the excised udder may be used in the study of milking by mechanical means and results of observations of the action of the mechanical milker on both excised and intact glands are reported.
2. When milk flows freely from the gland sinus into the teat there is no detectable vacuum created within the teat sinus.
3. When the intra-glandular pressure is sufficiently reduced the tissues become more flaccid and the teat cups crawl upward to cause a complete

closure of the orifice between the teat and gland sinuses. This fact accounts for the reported incomplete milking of some cows by machine.

4. Tugging upon the teat cups, when they begin to crawl, with sufficient force to bring them part way down on the teats will prevent closure of the passage and permits complete evacuation of all of the milk in the gland without resorting to hand milking.

5. When the teat cups have crawled upward to close the passage from the gland sinus to the teat the vacuum within the teat becomes identical to that in the milk line. It is postulated that this will have a traumatizing action upon the tissues being compressed. Attention is directed to the location of accessory secreting glands in the gland sinus and often in the upper part of the teat sinus. These are easily injured which predisposes to mastitis.

6. Good hand milkers apply a greater force to the teat with each squeeze than does the milking machine at ordinary recommended vacuums.

7. Observations of the action of the milking machine upon the teat and udder explains why milking speed is increased by stimulating the cow to let down milk before the milking is started.

8. It is concluded that when the mechanical milker is properly operated, especially removed as soon as the milk ceases flowing there is less danger of injury to the teat and udder than from hand milking.

#### ACKNOWLEDGMENT

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# THE EFFECT OF THYROIDECTOMY ON LACTATION IN THE BOVINE<sup>1</sup>

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Although the galactopoietic effect of thyroxine or dried thyroid gland is well established, reports on the effects of thyroidectomy are conflicting. A significant decrease in the milk yield of thyroidectomized goats was reported by Grimmer (5) and Trautman (14), while Hibbs *et al.* (6) obtained lactation for more than a year following thyroidectomy. Nelson and Tobin (11) and Nelson (10) have reported obtaining no evidence that lactation in thyroprived rats were impaired, while Folley (2) observed marked diminution of milk secretion and subnormal subsequent lactations. More recently Preheim (12) found only a slight decrease in lactation in the thyroidectomized rat, while Karnofsky (7) reported a marked reduction. For lactating cows, Graham (4) observed a slight decrease in milk production following thyroidectomy.

In view, therefore, of the conflicting reports of various workers, investigations were undertaken to obtain additional information regarding effects of thyroidectomy on lactation.

## PROCEDURE

Four grade Holstein females were selected for experimental subjects. Table 1 shows the age and previous history of each animal at the time of thyroidectomy.

TABLE 1  
*Experimental animals at time of thyroidectomy*

Animal No.	Age (months)	History
A26	13	Virgin
A23	16	46th day, 1st gestation
A15	36	12th day, 2nd lactation
E294	45	7th day, 2nd lactation

In order to assure complete removal of the thyroid, exploratory operations were performed on A26 and A23 ninety days after the initial operation. Autopsy examinations confirmed complete thyroidectomy of E294 and revealed incomplete removal from A15. Blood analysis, general appearance and behavior also indicated that A15 retained some functional thyroid tissue throughout the experimental period.

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Parathyroid tissue embedded in the thyroid was unavoidably removed; however, the accessory parathyroids were left intact. No effort was made to determine the extent of the remaining functional parathyroid tissue.

Environmental factors such as care, management, housing and feeding were minimized to some extent by maintaining the experimental animals under the same conditions as the regular dairy herd.

Daily milk yield was recorded and samples taken for analytical purposes at 14 day intervals insofar as conditions would permit. These samples were analyzed for fat, total nitrogen, casein nitrogen, lactose and specific gravity.

### RESULTS

The effects of incomplete and complete thyroidectomy on milk yield, fat yield and milk composition were studied. Data were accumulated on six lactations of four cows thyroidectomized at various stages of gestation and lactation.

*Incomplete thyroidectomy.* As shown in table 1 the thyroid glands were partially removed from A15 on the twelfth day of her first lactation period.

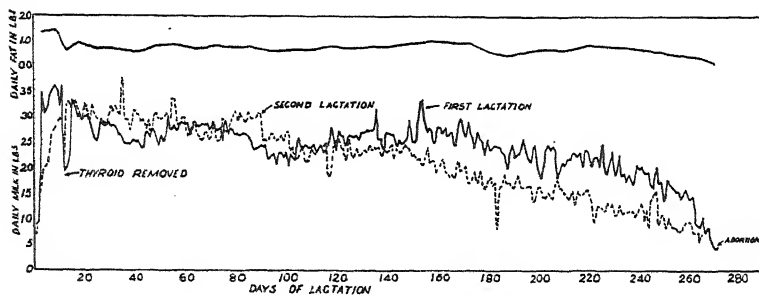


FIG. 1. Daily milk and fat yield of A15 following incomplete thyroidectomy on 12th day of first lactation. Second lactation began following abortion on 270th day of first lactation.

It is difficult to draw conclusions regarding the effects of incomplete thyroidectomy in view of the absence of previous lactations of A15 upon which to base a comparison. However, examination of the data presented in figure 1 reveals a distinct fall in milk production for 35 days following thyroidectomy, followed by a gradual upward trend of the curve of secretion. This response is undoubtedly explained, at least in part, by hypertrophy and hyperplasia of the remaining thyroid tissue which developed following the operation. The influence of thyroid hypertrophy is noticeably absent during the second lactation beginning after an abortion on the 270th day of the first lactation.

*Milk yield.* A comparison of the curves of milk secretion preceding and after thyroidectomy of E294, as shown in figure 2, clearly indicates the effects of thyroid ablation on milk yield. The point of maximum daily milk production was reached about 14 days previous to that of the normal lactation.

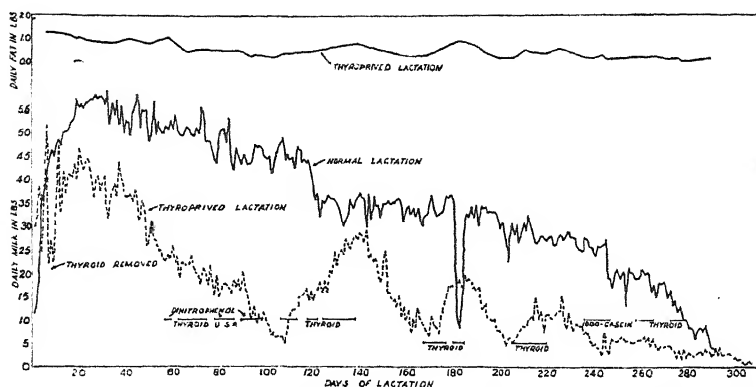


FIG. 2. Daily milk and fat yields of E294 for the lactation preceding thyroidectomy, for the subsequent thyroprived lactation, and during periods of thyrotherapy designated by —. Daily fat yields for prethyroprived lactation unavailable.

Milk secretion practically ceased after about 110 days while total milk yield was reduced about 70 per cent as compared to the previous lactations.

Milk secretion as affected by thyroidectomy during pregnancy is presented in figure 3. As shown in table 1, cow A23 was thyroidectomized on the 46th day of her first gestation which was of normal term. Unfortunately, two outbreaks of mastitis occurred during the lactation period; however, the general trend of the milk yield curve was not significantly altered. Daily milk production dropped to a low level after about 110 days and ceased entirely 183 days after parturition.

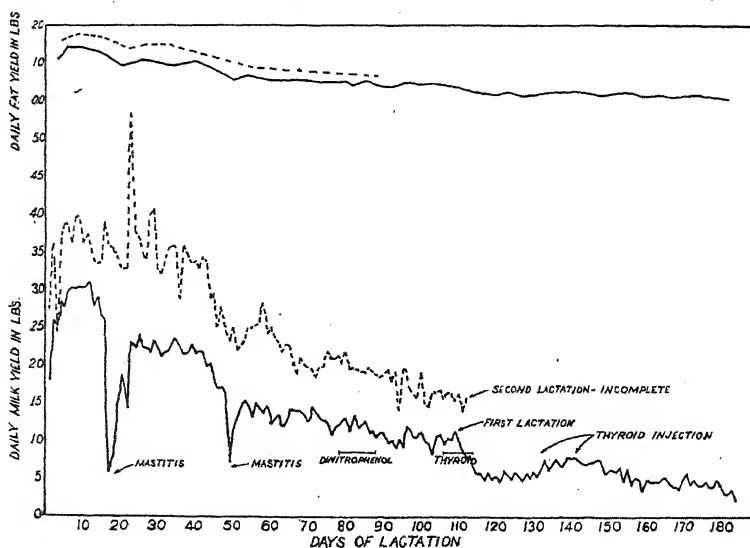


FIG. 3. First and second lactation milk secretion curves of A23 following thyroidectomy on 46th day of first gestation period.

Figures 1, 3, and 4 show the effects on milk secretion produced by removal of the thyroid glands before conception. The second lactation of A15 has been discussed. As shown in figure 3, A23 began her second lactation 720 days after thyroidectomy. A comparison of the second lactation and the preceding lactation indicates a higher level of secretion; however, the general trend of the curves is the same. Increased mammary development due to the beneficial effects of thyroid hormone or other factors from the developing fetus may account, at least in part, for the higher level of secretion observed during the second lactation. In this connection it is of interest to point out that a marked increase in skeletal growth of A23 was observed during the latter half of the preceding gestation period.

Figure 4 presents the milk secretion curve of A26 beginning 521 days after thyroidectomy. The curve of milk secretion is of normal shape; how-

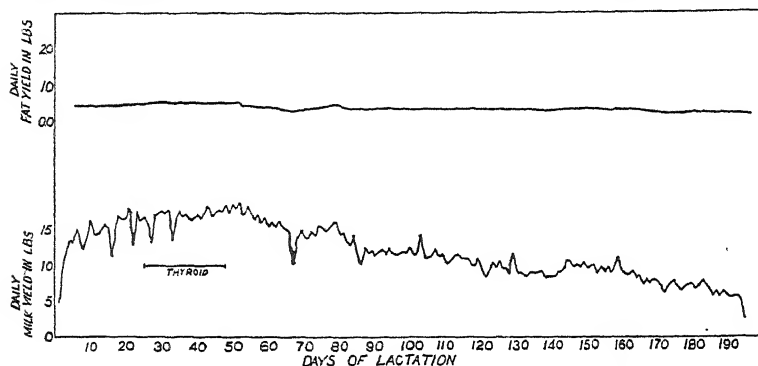


FIG. 4. Milk secretion curve of A26 beginning 521 days after thyroidectomy.

ever, lactation ceased 195 days after parturition. Although it is impossible to draw definite conclusions regarding the magnitude of the effects of thyroidectomy without previous knowledge of the producing ability of A26, it is significant that mammary development and milk secretion was possible some 521 days after thyroid removal.

*Fat yield.* The absence of more complete data on the daily production of milk fat precludes forming definite conclusions regarding the effects of thyroidectomy. It is, however, of interest to point out that based on the data obtained, the general trends and variations in fat yield were similar to those of milk yield.

*Composition of milk.* No significant changes in the lactose, total nitrogen and casein nitrogen content or specific gravity of the milk was evident from the results of periodical analyses. Values obtained for these constituents were all within the range for normal milk.

#### THYROTHERAPY

In view of the uniform decline of milk secretion observed after thyroidectomy, post-operative replacement therapy, in order to determine whether or



not some unknown non-endocrine factor was involved, seemed to be indicated.

*Dinitrophenol.* Dinitrophenol, administered orally to two thyroidectomized cows produced a variable response in milk and fat yield. Oral administration of 5 grams of dinitrophenol per day to A23 for 11 days beginning on the 78th day of lactation as indicated in figure 3 failed to evoke a response in milk secretion. The response of E294 to dinitrophenol administration was only slightly dissimilar, as shown in figure 2. During the period from the 78th day to the 87th day, 28 grams were orally administered producing an increase in milk yield of about four pounds per day. However, during the next period from the 89th to the 98th day, when 3.5 grams per day were administered, daily milk yield dropped from 16.6 to 9.5 pounds. As no calorimeter was available the relationship between metabolic rate, milk secretion and dinitrophenol administration is not known.

*Thyroid.* Fresh frozen thyroid glands were obtained from a local abattoir, mixed in a meat chopper, placed in gelatin capsules and administered orally. Desiccated thyroid, U.S.P. XI, was administered in two instances.

In order to ascertain whether or not thyroid deficiency was the sole limiting factor of maximum daily milk yield, 200 grams of thyroid were administered per day to A26 for 15 days beginning on the 23rd day of lactation. As indicated in figure 4, the response was negligible. This may be partially ascribed to progressive impairment of the normal physiological processes resulting in loss of ability to respond to thyroid therapy, as 546 days had elapsed since thyroidectomy.

Interpretation of results of thyroid therapy in the case of A23, figure 3, was complicated due to the presence of chronic mastitis.

The results of oral administration of fresh thyroid on milk and milk fat secretion are strikingly illustrated in figure 2. Fresh thyroid administered to E294 during six different periods, beginning on 106, 167, 198, 258, and 273 days of the lactation period, elicited a decreasing response with each trial until the increase in milk and milk fat yield was barely evident.

*Iodinated casein.* Turner (15) has presented evidence of the lactation-stimulating properties of iodinated casein. It was believed the availability of thyroidectomized cows presented an opportunity for testing the efficacy of this preparation. On the basis of response to previous administration of fresh thyroid, E294 possessed the ability to yield 12 to 14 pounds of milk per day. Oral administration of 3000 grams of iodinated casein over a period of 12 days resulted in only a slight increase in milk and milk fat yield. However, subsequent oral thyroid therapy failed to evoke a response; therefore, conclusions cannot be drawn regarding the lactation stimulating properties of iodinated casein.

In seeking an explanation for the decreasing response to thyrotherapy, progressive involution of the mammary gland with advancing lactation must

be considered. However, other factors may be involved, as involution of the mammary gland cannot account for the drastic decline and almost complete cessation of lactation 110 days after calving. Progressive impairment of normal physiological processes resulting in loss of ability to respond to thyroid therapy apparently occurs with the increasing interval of time between thyroidectomy and therapy.

#### DISCUSSION

Investigations on the rôle of the thyroid in lactation, using small laboratory animals, are hampered by the lack of suitable techniques for measuring milk yield, for microanalysis of milk, and the difficulty of achieving satisfactory and complete operative removal. These factors may partially account for the conflicting results of thyroidectomy studies reported in the literature.

Although control operations were not performed, post-operative recovery among the experimental animals, as indicated by daily milk yield, was uneventful and effected in about four days.

In view of the recent report of Folley *et al.* (3) that the failure of lactation in rats after thyroidectomy can be considerably alleviated by administration of parathyroid extract, cognizance must be taken of the parathyroids in thyroidectomy studies. Information regarding the location, number, and function of the parathyroid glands of the bovine is limited. Fehland (1) reported finding one parathyroid body embedded on the lateral surface of each thyroid lobe, and accessory parathyroids scattered along the course of the thyroid arteries. Although the amount of functional parathyroid tissue removed from or retained by each cow in this study was not determined, the absence of post-operative or parturitional tetany, characteristic of thyroidectomized rats, minimizes the importance of parathyroid insufficiency as a causative factor of decreased lactation. Normal blood calcium levels observed throughout the experimental period further substantiates the conclusion that our cows retained sufficient parathyroid tissue to suffice for normal calcium metabolism. Restoration of lactation by the administration of parathyroid extract to thyroidectomized cows would lend further proof.

Inasmuch as the administration of thyroid or dinitrophenol to thyroid-intact cows causes increases in fat content of the milk without necessarily affecting the quantity of milk, the absence of selective response of either milk or fat yield to thyrotherapy in this study is worthy of comment. These results may be explicable on the assumption that the metabolic level was lowered to such an extent that both milk and milk fat secretion would benefit from exogenous administration, whereas in the thyroid-intact animal selective response may occur. In this connection it is significant to point out that the milk of the thyroidectomized cows was of normal specific gravity and contained normal amounts of lactose, total nitrogen and casein nitrogen.

Results of replacement therapy strongly suggest that lactational failure observed in the bovine after thyroidectomy is due to thyroid insufficiency. However, the definite physiological effects of thyroid insufficiency causing decreased lactation are unknown. Subnormal mammary development in animals thyroidectomized a considerable period of time before pregnancy, as observed in this study, may be a factor. This observation is, however, contrary to those of Leonard and Reece (8) who reported increased mammary development after thyroid removal.

Karnofsky (7) and others, noting the changes in the pituitary gland after thyroidectomy, have postulated a decreased output of the lactogenic hormone as a limiting factor of lactation. McQueen-Williams (9) found that thyroparathyroidectomized male rat pituitaries contained less lactogen than normal. However, Reineke *et al.* (13) report both the lactogenic and thyrotropic hormones present in the pituitaries of thyroidectomized male kids in normal concentrations. Restoration of lactation to normal in thyroidectomized animals by administration of prolactin would confirm this postulation.

Milk secretion is dependent upon an adequate supply of metabolites from the blood. A decreased supply of metabolites may be due to lower levels in the blood stream or to a decreased flow of blood through the mammary gland. A slight reduction of heart rate in the thyroidectomized cows was observed. Reineke and co-workers (13) have suggested a reciprocal relationship between the thyroid and pituitary whereby removal of the thyroid results in a decrease of the pituitary factors regulating the metabolism of sugar, fat, protein, and mineral matter. Only in their presence can large amounts of milk be made. Normal levels of blood sugar, total nitrogen, calcium, phosphorus, and fat were observed throughout the experimental period for the thyroidectomized cows in this study.

Considerable stress is placed upon general metabolism during lactation. Inasmuch as thyroidectomy causes a lowering of metabolic activity this is undoubtedly a contributing cause of the rapid decline of milk secretion after thyroidectomy. Whether or not thyroidectomy results in a distinct lowering of metabolic activity of the individual secretory cells of the mammary gland remains a moot question.

#### SUMMARY

1. Thyroidectomy performed on cows preceding gestation, during pregnancy, and during lactation caused a complete cessation of lactation in about 180 days.
2. Total milk and fat yield were reduced about 75 per cent.
3. Incomplete removal of thyroid gland produced a temporary decline in milk secretion followed by a gradual return to former levels.
4. Composition of the milk as determined by fat, lactose, nitrogen, and specific gravity analyses was not affected.

5. Milk and fat yields were restored to former levels by oral administration of fresh thyroid. Decreasing response was observed as the lactation period progressed.

6. It is concluded that the reduction of lactation after thyroidectomy is due mainly to thyroid deficiency.

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# THE EFFECT OF COMPLETE EVACUATION OF THE MAMMARY GLAND BY PITOCIN UPON MILK AND FAT PRODUCTION\*

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It is generally recognized that incomplete removal of the milk from the udder over a period of time will cause a decline in lactation. Partial removal of the milk has been used by many as a means of "drying off" cows. Miller and Petersen (4) observed marked downward trends in the lactation curve when cows were stimulated to "let down" their milk 20 minutes before milking began, resulting in incomplete evacuation of the gland.

While the reason for this effect of incomplete evacuation of the gland is speculative at the present it can best be explained by increased intra-alveolar pressures created by the retained milk. Petersen and Rigor (5) and Garison and Turner (3) showed a decreased rate of milk secretion with increased pressures. The former observed complete stoppage of milk secretion when these pressures reached 30 mm. Hg while the latter noted that 40 mm. Hg pressure was needed to completely stop milk secretion. The increased milk production observed with increased frequency of milking can also be explained by the hypothesis that this practice prevents the development of as great pressures or the maintenance of high pressures over as long periods of time as does less frequent milking.

Since it has been established that incomplete evacuation of the udder over a period of time will cause a drop in the lactation curve, the question arose as to what part natural incomplete emptying of the gland plays in the decline of lactation with the advance in lactation. A second question is that of the effect upon the lactation curve of cows that are erratic in their let down of milk and if incomplete let down of milk might not be the cause of the rapid decline in the lactation observed in many cases.

As the oxytocic principle (2), when injected intravenously, has been shown to practically completely evacuate the alveoli, use of this hormone following milking enables one to ascertain the amount of milk remaining in the gland after a normal milking has been completed. Complete evacuation of the gland at each milking, by use of this hormone, will also make it possible to determine the effect of this procedure upon the lactation curve.

## EXPERIMENTAL

To determine the effect of complete evacuation of the gland at each milking upon the lactation curve in the declining phase of lactation 5 cows in

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advanced stages of lactation were used. The cows were mature grade Holsteins designated as E 293, E 366, A 12, A 15, and A 30. They were all normal during the period of the experiment.

The experiment was divided into three periods of fourteen days each. For E 293 and A 15, injections were made in the second period and the first and third periods served as controls. E 366, A 12, and A 30 were injected during the first and third periods and the second period served as a control.

All cows were milked twice daily at twelve-hour intervals. E 366 was milked by hand and the others by machine. In the case of machine milking completeness of milking was checked by hand stripping. The milk obtained by hand or machine plus any stripping is referred to as normal milk.

During the injection period 1 cc. Pitocin containing 10 I.U. of the oxytocic principle was injected intrajugularly immediately after the completion of the normal milking. The milk obtained following the injection, referred to in this experiment as "pitocin" milk, was weighed and analyzed separately.

In addition to the weights of milk obtained at each milking, lactose (1) chloride (6) and fat determinations were made.

The effect of complete evacuation of the udder by injection of Pitocin was studied on three cases that deserve special mention.

Case 1. A grade first-calf Holstein heifer that apparently did not let down her milk.

Case 2. A purebred Jersey in a nearby herd in the third month of her second lactation period, with a short time lactation history that could not be explained on a basis of heredity.

Case 3. A purebred Jersey cow in the fourth lactation that was unusually erratic in the let down of her milk.

#### RESULTS AND DISCUSSION

A summary of the actual milk and fat production and fat percentage by periods for both the "normal" and "pitocin" milks is presented in table 1. It is customary in experiments with the setup used here to calculate the effect of the experimental procedure by comparison of the experimental with the control periods. This has been done in table 1 but caution should be registered in accepting those values as representing the absolute effect of complete evacuation of the gland by Pitocin because nothing is known about the effect of the injections upon subsequent periods and all cows were in the declining phase of lactation with no means of knowing what the normal slope of the lactation curve would be. With the exception of A 30 the evidence seems to be in favor of the Pitocin injections causing a greater increase in production than indicated by the calculations. Because of the injections the subsequent control period seems to be at a higher level than it would have been normally. It can therefore be said that in every case

the complete evacuation of the udder by Pitocin injections causes a significant increase in the amount of milk produced.

The effect of the Pitocin injections upon the fat percentage is not so conclusive although the indications are that this value is also increased. In three of the cows there is the significant increase of fat percentage of 0.4+ while the decreases are 0.08, in both cases. It is to be noted that the "pitocin" milks are significantly higher in fat content than the "normal" milks. It is also noteworthy that the fat content of the "normal" milks during the injection periods are significantly lower than for the control periods.

TABLE 1

*The effect of complete evacuation of the udder by Pitocin injections. Total milk and milk fat production and fat percentage by periods of 14 days each. For comparative purposes the milk production for a fourteen-day period previous to the start of the experiment was taken from the daily milk records. This is designated Control +*

Cow number	Experi- mental periods	Milk produced during 14-day periods						Period of in- jection as com- pared to control	
		Normal milking		Pitocin milk		Total			
		Milk	Fat	Milk	Fat	Milk	Fat	Lbs.	% fat
E 293	Control+	lbs.	%	lbs.	%	lbs.	%		
	Control	399.9	3.29	.....	.....	399.9	3.29	.....	.....
	Injection	320.2	2.98	99.7	6.31	419.9	3.77	+ 62.2	+ 0.42
A 15	Control	315.5	3.41	.....	.....	315.5	3.41	.....	.....
	Control+	.....	.....	.....	.....	435.7	.....	.....	.....
	Control	386.9	3.49	.....	.....	386.9	3.49	.....	.....
E 366	Injection	315.0	2.86	105.1	6.68	420.1	3.82	+ 45.8	+ 0.40
	Control	361.7	3.35	.....	.....	361.7	3.35	.....	.....
	Control+	.....	.....	.....	.....	456.4	.....	.....	.....
A 30	Injection	363.6	2.84	102.0	7.16	465.6	3.79	.....	.....
	Control	410.2	3.34	.....	.....	410.2	3.34	+ 67.1	+ 0.43
	Injection	370.9	2.54	118.0	7.54	488.9	3.75	.....	.....
A 12	Control+	.....	.....	.....	.....	245.3	.....	.....	.....
	Injection	229.0	3.58	24.1	7.68	253.1	3.97	.....	.....
	Control	234.5	4.06	.....	.....	234.5	4.06	+ 15.0	- 0.08
A 12	Injection	217.1	3.56	28.7	7.20	245.8	3.99	.....	.....
	Control+	.....	.....	.....	.....	149.3	.....	.....	.....
	Injection	68.0	3.35	81.0	7.14	149.0	5.41	.....	.....
A 12	Control	31.7	5.39	.....	.....	31.7	5.39	+ 59.8	- 0.08
	Injection	19.9	3.37	14.0	7.81	33.9	5.20	.....	.....

While the production of both milk and fat in the experimental periods exceed that of the control periods by significant amounts for E 293, A 15, E 366, and A 30; for A 12 the production during the experimental period only equaled that of the control periods. This can be explained by the fact that she was in the last stage of the lactation and the drop in daily production was precipitous when the experiment was started.

The lactose and chloride contents of the milk were unaffected by Pitocin injections.

To ascertain the effect of Pitocin injections with time the total daily milk and fat production for all periods were plotted. For the injection

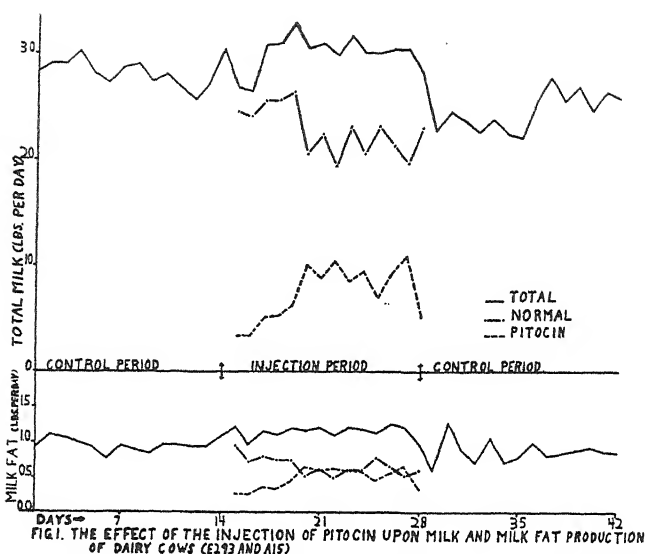


FIG. 1. THE EFFECT OF THE INJECTION OF PITOCIN UPON MILK AND MILK FAT PRODUCTION OF DAIRY COWS (E 293 AND A 15)

FIG. 1. The effect of complete evacuation of the udder by administration of Pitocin at each milking upon milk and milk fat production. The average daily milk and milk fat is plotted for E 293 and A 15.

periods the "normal" and "pitocin" milks and fats are also plotted. Because of the similarity of the curves these records of E 293 and A 15 are combined in figure 1 and for the same reason the records on E 366 and A 30

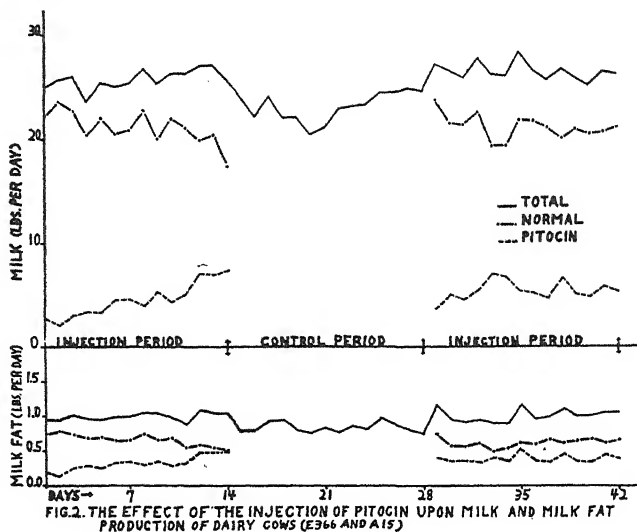


FIG. 2. THE EFFECT OF THE INJECTION OF PITOCIN UPON MILK AND MILK FAT PRODUCTION OF DAIRY COWS (E 366 AND A 30)

FIG. 2. The effect of complete evacuation of the udder by Pitocin administration upon the average daily milk and milk fat production on E 366 and A 30.



are combined in figure 2. The record of A 12 presenting a different picture is presented in figure 3.

As the injections continued during the 14-day periods, there was an upward trend in both total daily milk and fat production in spite of the fact that all of the cows were in decided declining phases of lactation. This trend is relatively the greatest in the second injection period in A 12 where also the drop was the most precipitous in the control period following the first injection period. This fact is taken as evidence that milk retained in the gland after normal milking is a contributory factor to the decline in production in late lactation. On the basis of the results on A 12 there is indication that "drying off" effect of the retained milk is the greatest in the most advanced lactation.

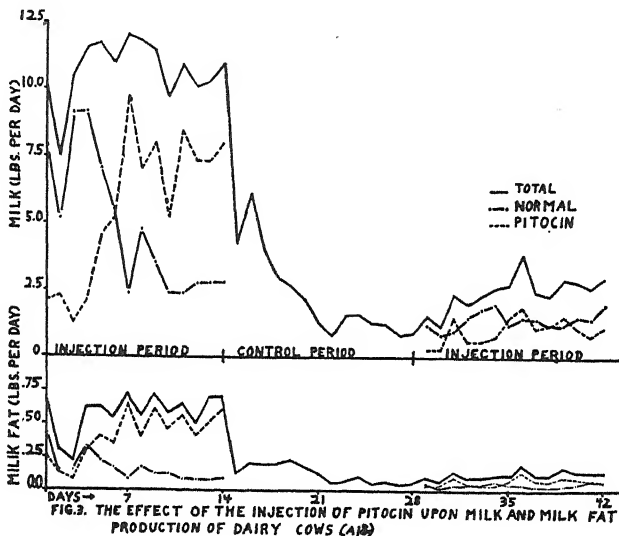


FIG. 3. The effect of complete evacuation of the udder by Pitocin administration upon the daily milk and milk fat production on A 12.

Another interesting observation is that as the injections continued there was a progressive decrease in the "normal" milk obtained and an increase in the "pitocin" milk and the shift in the ratio of the fat in the "pitocin" milk to that in the "normal" milk is even greater with the continuation of injections. This effect of Pitocin injections is the most prominent in the first injection period of A 12 (fig. 3) where in the whole period 68.0 pounds were obtained as normal milk and 81.0 pounds as "pitocin" milk while in the second half of the period the "pitocin" milk amounted to more than 3 times as much as the "normal" milk.

The reason for the observed phenomenon can only be speculative at this time. It is possible that the cows were becoming conditioned to the injec-

tions and did not respond as completely with the let down of milk to the normal milking. An alternate explanation is that the continued injections had a depressing effect upon the natural secretion of the oxytocic principle by the posterior pituitary body.

The results obtained by Pitocin injections into the 3 special cases investigated, adds confirmatory evidence to the depressing effect upon lactation of incomplete evacuation of the mammary gland even though such be from natural causes. Since each of the 3 cases present essentially different aspects of the problems they will be dealt with separately.

Case 1 can best be described as a first calf heifer that lacked the ability to respond with a "let down" of milk to the normal milking stimulus. The only milk obtained was that which had drained into the lower cavities of the udder and in the teats. She exhibited no nervous signs during attempts at milking and at no time could more than 3.1 pounds of milk be obtained at a milking in spite of the fact the size and appearance of the udder indicated a much greater producing capacity. After the small amount of milk was removed the lower part of the udder became "soft" but the upper two-thirds remained firm. After two weeks 1 cc. Pitocin was injected intrajugularly immediately after each milking and the additional milk removed. This procedure was continued for five days. During the injection period she averaged 16.2 pounds per milking of which 13.9 pounds were obtained following the injection of the hormone.

Upon cessations of the injections no more milk was obtained than during the first two weeks and at the end of two months milk secretion ceased. After evacuation of the gland following the injections it assumed the natural soft texture following the milking of an udder said to have good quality. The hardness of the gland was therefore due to the retained milk.

Case 2 differed from Case 1 in that she milked at normal levels after parturition but dropped off in production at a much more rapid rate than any of her closely related females in this herd. The case came to the attention of one of the authors when the owner explained the reason for the lack of persistency as being due to a "meaty" udder. She was then in the third month of lactation and was producing 9.5 to 10.3 pounds of milk per milking. After removal of this amount of milk the upper two-thirds of the udder remained hard or "meaty" as the owner had described.

Upon the intrajugular injection of 1 cc. Pitocin immediately after a normal milking of 10.1 pounds an additional 8.6 pounds of milk was obtained. Following the removal of this amount of milk the hardness or meaty condition of the udder had disappeared. Injections were continued by the owner for a period of 5 days with a reported increase of more than 50 per cent production. Upon cessation of injections she immediately returned to the former habit of incomplete evacuation of the gland.

As the amounts of milk obtained were far greater than the capacities of the cavities of the udder and teats and also as the amounts of milk obtained at milkings varied no more than could normally be expected it appears that in this case there was a uniform but incomplete response to the milking stimulus. Whether the lack of response was due to insufficient hormone secretion or to a decrease in the sensitivity of the gland musculature is conjectural.

Case 3 is a pure bred Jersey in the University herd now in her fourth lactation. Her history is of special interest and therefore her lactation curves for the first 3 completed and the 4th lactations to the present are given in figure 4. It will be noted that in her first lactation she was unusually persistent but in the subsequent ones the reverse is true.

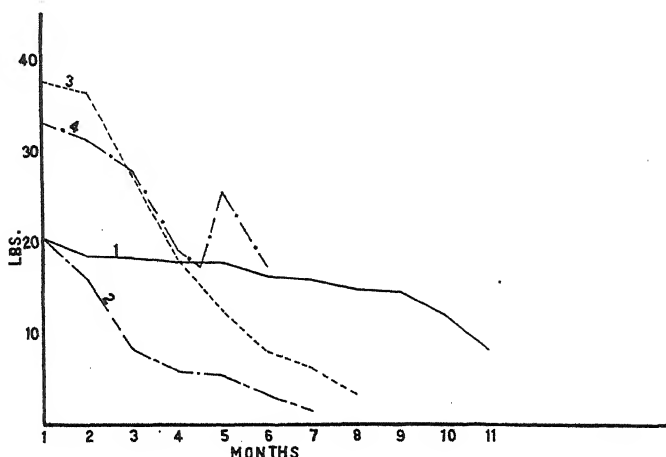


FIG. 4. The effect of erratic habit on let down of milk upon the character of the lactation curves, plotted by monthly averages except the portion of the fourth lactation where plotting is based upon weekly average. Successive lactations labeled 1, 2, 3, and 4. During the first lactation complete let down occurred at each milking.

Inspection of the daily milk records reveal that just before a marked decline appears in the lactation curves she becomes very irregular in the amount of milk obtained. It was not uncommon to obtain but two or three pounds of milk in a milking to be followed by one of fourteen to sixteen pounds. The variations in the month of her fourth lactation may be seen in figure 5. Following the milkings of small amounts the upper part of the udder remained hard while following the large milking it was soft and pliable. Inspection of the curve (fig. 5) for the first fourteen days reveals the probability that at some milkings there was apparently no response with a let down of milk while at others there was a partial response and sometimes a complete response. It is safe to say that the gland was completely

evacuated in less than one-third of the milkings, which fact was responsible for the rapid decline in her lactation curve.

For fourteen days (14 to 28 in figure 5) 1 cc. Pitocin was injected intrajugularly immediately after each normal milking and the milk let down removed. The milk obtained following Pitocin injection as well as the total milk for each milking is plotted in figure 5. It will be noted that the variations in the total milk obtained per milking was reduced to minor fluctuations. There were, however, marked variations from milking to milking in the relative amounts obtained as "normal" and "pitocin" milk.

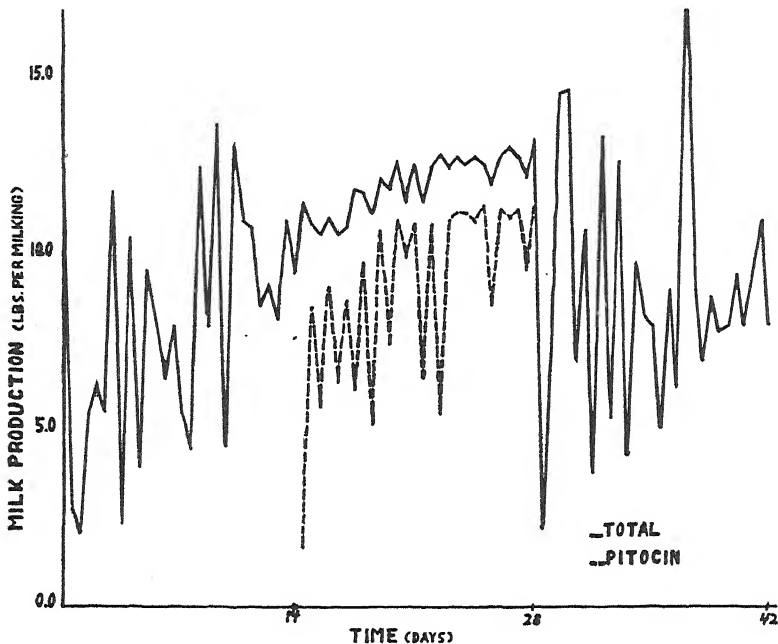


Fig. 5. The effect of complete evacuation of the udder by Pitocin injection upon the quantity of milk and uniformity of amounts of milk in a cow with erratic milking habits. Each milking is plotted. The first fourteen days is a control period. Pitocin was injected at each milking from the 15th to 28th day.

Using the milk production for the fourteen days immediately preceding the injection period there were increases of 44.8 per cent in milk and 59.31 per cent in fat for the injection period. It will be noted from figure 5, however, that the daily production increased for the first 8 days when a plateau is reached. Using the last six days of the injection period the production increased 56.9 per cent as compared with the average production for the fourteen days before injection.

As for the injections of Pitocin in cows in advanced lactation it is noted there is an increase in "pitocin" milk and a decrease in "normal" milk with

continuation of the injections. It is noteworthy that the decreasing effect of the Pitocin injections upon the amounts of milk lasted for but one milking after injections were stopped. It is also noteworthy that in the 14-day period following cessation of injections the milk production is 5.1 per cent greater than in the 14-day period preceding injections instead of an estimated 15 per cent loss as would be expected from the trend of the lactation curve.

It would therefore appear that the complete evacuation of the gland at each milking by the use of Pitocin not only checked the downward trend of the lactation curve but in this case stimulated the gland into greater activity.

#### SUMMARY AND CONCLUSIONS

1. The results are given of complete evacuation of the udder by the injection of Pitocin upon the milk and fat production of five cows in the declining phase of lactation and of three other cases of natural incomplete let down of milk.
2. In all cases of declining lactation complete evacuation of the gland checked the downward trend of the lactation. In all but one case the milk production was significantly increased over the control period.
3. On the basis of the results obtained on three cows with the evacuation of the gland by the injection of Pitocin it is suggested that in many cases the lack of persistency is due to the incomplete let down of milk.
4. Any milk retained in the gland has a depressing influence upon subsequent production.

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# CHANGES IN BACTERIAL COUNTS OF STORED ICE CREAM MIX

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In ice cream surveys conducted by this station excessively high bacterial plate counts sometimes were obtained on samples frozen from mixes which originated at plants whose products ordinarily were of low bacterial content. Although sanitation in the freezing plant undoubtedly was a factor in some instances, other causes were indicated in a sufficient number of cases to warrant study of the effect of time and temperature of storage upon the development of bacteria in ice cream mix.

Abele (1) reported only 80 per cent of the mix in the hands of the manufacturers and 75 per cent of that in possession of the freezers was held below 50° F. Temperatures of ice cream mix in transit commonly were found to rise above 50° F., and sometimes to 60° F., accentuating the problem of adequate refrigeration during storage in the plant where the mix was frozen.

## METHODS

Samples of pasteurized mix were obtained from two sources and at different seasons of the year over a period of 15 months. During this period ingredients and formulae were changed, especially at one source, so the samples represented a diversity of composition. Each original lot of mix was agitated thoroughly and approximately 50-ml. quantities transferred aseptically to 2-oz. screw-cap sample jars. A number of replicate samples sufficient to provide an undisturbed sample from each temperature of storage at each plating interval was employed. The samples were held under commercial refrigeration conditions, periodic temperature readings being recorded. On some samples the temperatures may have exceeded the recorded limits for short unrecorded intervals, but the ranges given represent the temperatures in effect during most of the holding period. All platings were made by using the volumetric method, standard plate counts being on Tryptone-glucose-extract-milk agar incubated for 48 hours at 37° C., and coliform counts being on violet-red bile agar incubated approximately 20 hours at 37° C. The procedures used were those outlined in "Standard Methods for the Examination of Dairy Products" (2).

## RESULTS

The results of the bacteriological examinations made after various intervals are recorded in table 1. The data indicate some lots of mix cannot be

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TABLE 1

*Changes in bacterial counts of ice cream mix during refrigerated storage*

Sample No.	Holding temperature range (°C.)	Standard plate count per ml. after holding for periods indicated			
		0 days	2 days	4 days	7 days
1	2.0-10.0	9,300	4,200	17,000	28,000
	13.0-14.5	9,300	10,000	1,500,000	75,000,000
2	4.5- 5.5	14,000	13,200	9,100*	11,700
	7.0- 8.0	14,000	19,000	9,800*	16,000
3	4.5- 5.5	41,000	92,000	178,000*	3,400,000
	7.0- 8.0	41,000	185,000	8,800,000*	68,000,000
4	4.5- 5.5	2,500	4,500	2,300	23,000†
	5.5- 8.5	2,500	6,300	280,000	19,000,000†
	12.8-14.5	2,500	9,700	1,500,000	47,000,000†
5	3.0- 4.5	9,200	9,800	40,000	470,000
	8.5- 9.5	9,200	35,000	920,000	30,000,000
	12.2-13.3	9,200	78,000	4,200,000	135,000,000
6	3.0- 4.5	15,000	6,100	6,600	51,000
	8.5- 9.5	15,000	7,100	150,000	9,500,000
	12.2-13.3	15,000	10,000	1,300,000	57,000,000
7	3.0- 4.5	6,600	4,600	11,500	116,000
	8.5- 9.5	6,600	8,700	180,000	5,400,000
	12.2-13.3	6,600	13,400	940,000	16,100,000
8	1.0- 4.5	3,700	6,100	9,200	20,000,000
	4.0- 5.0	3,700	4,500	24,000	53,000,000
	14.3-15.5	3,700	17,000	510,000	240,000,000
9	3.0- 4.5	20,000	59,000	66,000	156,000
	8.0- 9.5	20,000	115,000	7,000,000	61,000,000
	12.2-14.0	20,000	220,000	13,000,000	97,000,000
Coliform count per ml.					
1	2.0-10.0	.....	.....	.....	.....
	13.0-14.5	.....	.....	.....	.....
2	4.5- 5.5	1	1	0*	7
	7.0- 8.0	1	2	13*	5
3	4.5- 5.5	320	380	380*	700
	7.0- 8.0	320	920	3,400*	18,100
4	4.5- 5.5	1	1	< 1	1†
	5.5- 8.5	1	1	7‡	4,900†
	12.8-14.5	1	6	> 100	710,000†
5	3.0- 4.5	> 100	370	450	320
	8.5- 9.5	> 100	450	410	160,000
	12.2-13.3	> 100	500	11,000	270,000
6	3.0- 4.5	> 100	440	380	220
	8.5- 9.5	> 100	590	630	500
	12.2-13.3	> 100	800	900	1,500
7	3.0- 4.5	1	1	1	300†
	8.5- 9.5	1	4	7	420
	12.2-13.3	1	6	420	500
8	1.0- 4.5	< 1	< 1	< 1	< 1
	4.0- 5.0	< 1	< 1	< 1	< 1
	14.3-15.5	< 1	< 1	< 1	< 1
9	3.0- 4.5	120	70	10	130
	8.0- 9.5	120	350	16,000	320,000
	12.2-14.0	120	700	59,000	1,600,000

\* = 5 days.    † = 8 days.    ‡ = Questionable coliform types.



held more than 4 days at temperatures slightly below 5.5° C. (42° F.) without significant increase in count. No relationship between initial coliform count and tendency for standard plate count to increase at this temperature is apparent. In some instances relatively high counts were obtained after holding the mix 7 days at 5.5° C. (42° F.) or below. Temperatures of storage ranging up to 9.5° C. (49° F.) commonly resulted in some increase in standard plate count after 2 days and a large increase in count after 4 days. Holding mix at this temperature for 7 days usually resulted in excessive counts. Holding at temperatures approaching 15.5° C. (60° F.) sometimes permitted considerable increase in standard plate count in 2 days and always resulted in excessively high counts after 4 days.

Development of coliform organisms was checked by holding temperatures of 5.5° C. (42° F.) or below. At temperatures approaching 9.5° C. (49° F.) the behavior of this group of organisms was erratic, considerable increases in count being encountered in some samples, while these organisms multiplied but little or not at all in this temperature range in other samples. Storage at temperatures of 12.0° C. (53.6° F.) and above resulted in marked increases in coliform count, even after 4 days.

In no case had the flavor or aroma of the mix been changed significantly during the holding period of 7 or 8 days.

#### DISCUSSION

The data indicate storage of mix initially having a low bacterial count may permit considerable increases in "total" count. These increases are of particular significance when temperatures above 5.5° C. (42° F.) are encountered. Increases in coliform count usually occurred when temperatures reached the level of 8.0° C. (46.4° F.) or above, but were not significant below this temperature level. Increases in numbers of these bacteria during storage could result in erroneous conclusions concerning magnitude of post-pasteurization contamination of the mix.

The data indicate the practice of making or purchasing mix and storing it under moderate refrigeration for several days before freezing may have an undesirable effect upon the bacteriological condition of the resulting ice cream. Hammer (3) showed more than 30 years ago that the bacterial content of frozen ice cream fails to increase, in fact usually decreases, during storage. Storage in the form of frozen ice cream, rather than in the form of unfrozen mix, is to be preferred in either the manufacturing or the freezing plant. If the temperature can be maintained below 40° F., storage of mix for not more than 2 days apparently would cause no bacteriological difficulties under usual conditions.

#### CONCLUSIONS

1. The bacterial count of ice cream mix stored at 4.5° C. (40° F.) or

above increases with storage time and may reach considerable magnitude as the storage temperature increases.

2. Coliform bacteria usually were found to increase in numbers in ice cream mix held at temperatures of 8.0° C. (46.4° F.) or above. This increase may give rise to false conclusions relative to post-pasteurization contamination based upon presence of coliform bacteria in appreciable numbers.

3. Storage of frozen ice cream, rather than unfrozen mix, is recommended.

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## A SIMPLIFIED METHOD OF ESTIMATING 305-DAY LACTATION PRODUCTION\*

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Records of 305-day milk and butterfat production are ordinarily estimated from monthly milk weights and butterfat tests. Probably the most widely used method is that recommended by the Division of Dairy Herd Improvement Investigations, Bureau of Dairy Industry. The D.H.I.A. method has become known as the centering method because the testing month is centered around an established testing day rather than coinciding with the calendar month. In addition to this, the method involves calculation of back-credit in cases of lactation beginning at certain times in the testing month. In an investigation using D.H.I.A. records many errors in calculation were found. The majority of these errors were made in calculating the back-credit period, but many others resulted from the considerable multiplication and addition involved. This means that unless much time is spent in checking records, selection of animals is likely to be based on inaccurate information. Any method which might be substituted for the present D.H.I.A. system should be simple enough to result in greater arithmetical accuracy and should yield a record which possesses those statistical properties important in genetic selection.

The summation of the first ten testing-day values multiplied by 30.5 suggests itself as a simple method which should result in less arithmetical error. In this study the statistical properties of records calculated by this simplified method have been compared with the same cows' 305-day production records computed by the D.H.I.A. centering method and with actual production records obtained by summing the daily milk weights for the first 305 days.

### LITERATURE

Prior to the advent of the cow testing associations, producing ability in dairy cattle had been estimated from records based on weekly butter yield and daily milk weights. Other estimates were based on seven-day tests in the fourth month multiplied by the number of weeks in the lactation; and still others by adding the milk weights for three days per month over a 12-month period and multiplying this sum by 10 to estimate yearly production.

Since cow testing associations were established, records have been calculated in most cases by the D.H.I.A. centering method. In some cases, how-

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ever, a calendar month scheme which is based on monthly tests and the number of days the cow is milked during the month has been used, as well as another method which estimates the production for the period between two consecutive testing dates from the milk and butterfat yields of the later date. Still other estimates have been derived from daily milk weights and a monthly or bimonthly fat test, and Plum (8) has used the summation of the first eight testing-day yields in an investigation of the causes of differences in butterfat records.

Studies by Rabild (9), McCandlish and McVicar (5), McDowell (6), Copeland (1), Gifford (2), Kendrick (4) and McKellip and Seath (7) indicated that little difference exists between the averages of production records estimated by these various methods. Harris, Lush and Shultz (3) found no significant differences in repeatabilities of D.H.I.A., lactation and yearly production records.

#### MATERIALS AND METHODS

The milk and butterfat figures used in this study were obtained from the D.H.I.A. herd record books and the daily milk records of two Wisconsin State Department of Public Welfare herds. These figures represented the production between 1935 and 1941 of Holstein cows milked three times daily. In one herd (Herd I), the butterfat records on 257 lactations of 108 cows and the milk records on 160 lactations of 60 cows were used. In the other herd (Herd II), the study was based on the milk records from 81 lactations of 65 cows. The milk records calculated by the D.H.I.A. centering method and simplified method were compared with each other and with the actual production figures obtained by summing the first 305-days' milk weights. Only the former comparison, "centered" vs. "simplified," could be made with the butterfat records.

Differences in the means of the records were tested for significance using Student's "t" test for paired observations. Simple correlations between estimated and actual yields were calculated. The variances of the milk and butterfat records, calculated for each herd by both methods, were divided into portions due to cow differences and differences between records of the same cow. The repeatability of milk and butterfat estimates (correlation between records made by the same cow) was computed for each class of records, *i.e.*, for the simplified, centered and actual production figures.

#### RESULTS AND DISCUSSION

The analyses show that the simplified and centered estimates are highly correlated with each other for both milk and butterfat production, and that the actual milk records are also closely associated with the two estimates of milk production (tables 1 and 2).

The means of the centered records for butterfat and milk production are significantly higher than the averages of the simplified records in Herd I

TABLE 1

*Correlations between milk records calculated by the simplified and centering methods and actual yield in Herds I and II*

Herd	I		II	
	Simplified	Centering	Simplified	Centering
Actual .....	0.990	0.992	0.984	0.991
Simplified .....	.....	0.995	.....	0.984

(tables 2 and 3). These higher means are probably caused in large part by the back-credit additions used in the calculations of centered records. The tester tests all milking cows that are more than six days beyond calving at

TABLE 2

*Statistics of butterfat records calculated by the simplified and centering methods in Herd I*

Method	Number of records	Mean butterfat production	Standard deviation	Mean difference	Correlation between	
					Simplified and centered records	Intra-cow records
Simplified ...	257	507.8 ± 6.3	101.2	3.4*	0.993	0.24
Centering .....	257	511.2 ± 6.3	100.8			0.25

\*  $P < 0.01$ .

the time of his visit. Cows which both freshen and are tested during a testing period are given credit for the portion of the testing period they should lactate, less three days. Cows calving during an immediately previous testing period and not tested within that period are given credit (back-

TABLE 3

*The actual, simplified and centered mean milk yields and differences between these means in Herds I and II*

	Number of records	Actual	Simplified	Centering
Herd I .....	160			
Mean yield .....	.....	14494 ± 207	14600 ± 210	14727 ± 212
Difference .....	.....			
Actual .....	.....	.....	106*	233*
Simplified .....	.....	.....	.....	127*
Herd II .....	81			
Mean yield .....	.....	12683 ± 234	12769 ± 238	12565 ± 234
Difference .....	.....			
Actual .....	.....	.....	86†	118*
Simplified .....	.....	.....	.....	204*

\*  $P < 0.01$ .

†  $P \approx 0.03$ .

credit) for the portion of the lactation which occurred in the previous period, less three days, on the basis of production during the present testing period. At each test (subsequent to the first) in the lactation of a cow, production is calculated for the testing period from the testing-day figures multiplied by the number of days in the testing period.

In a sense there are two groups of cows, one in which the cows start their records by receiving back-credit and another in which they do not. At the end of the record the "back-credit" cows will use only a part of the tenth testing period to complete a 305-day period. For the "non-back-credit" cows a portion of the eleventh testing period must be added to obtain an estimate of production over a 305-day period.

The simplified scheme also requires that each cow tested must have calved six or more days previous to the tester's visit, but no correction is made for day of calving (each testing-day value being multiplied by 30.5). Hence the simplified calculations give equal weight to the production on all of the first 10 testing periods, whereas the back-credit calculations give more weight to the higher production of the first testing period and less weight to the lower production of the tenth testing period than does the simplified scheme. Therefore, in "back-credit" lactations, the centered records are larger than those determined by the simplified method. On the other hand, cows receiving no back-credit will have lower centered records than simplified records because only a portion of the first testing period is used (the lower production of the eleventh period being used to complete the record), while in the simplified calculations equal weight is again given to all of the first ten monthly tests.

Furthermore, in the centering method the chances of cows receiving and not receiving back-credit are unequal. This grows out of the fact that a lactation record begins on the fourth day of lactation, whereas a test cannot be made until the seventh day of a lactation. Three days thus are added to the portion of the month in which a cow may calve and receive back-credit (average for different months of the year approximately  $14\frac{1}{2}$  out of  $30\frac{1}{2}$  days), and likewise the portion of the month in which they should not receive back-credit (about 16 out of  $30\frac{1}{2}$  days) is lessened by three days. The two periods therefore approximate  $17\frac{1}{2}$  and 13 days respectively. Thus the average of many centered records can be expected to be higher than the mean of corresponding simplified figures.

The simplified estimates of milk production in Herd II, on the other hand, are significantly higher than the centered records. The difference between Herd I and Herd II in these results seems to lie in the difference between these herds in regard to the deviations of actual testing dates from the established centering-day (table 4). In Herd I the deviations between the testing dates and centering-day are small, whereas the deviations in Herd II are large, varying from seven days before to 23 days after the centering-day.

Since the actual testing dates in Herd II were usually later than the fixed centering-date (ave. = +7.8 days), the testing days were near the end of the testing period. This means that cows which would have received back-credit (*i.e.*, freshened after the seventh day before the tester's visit) if the actual testing date had been closer to the centering-day would be included in the first testing period of that lactation, and therefore receive no back-credit. The increase of non-back-credit lactations in this herd undoubtedly explains why the centered records are lower than the simplified records in Herd II (table 3).

The means of the simplified estimates are significantly larger than the means of the actual milk yields in Herds I and II (table 3). One explanation for this is that the first test is frequently made at a time when the cow is producing at a higher rate than the average for that part of the first 30.5-day period in which she is actually in production. In addition, cows tested soon after freshening are given credit for production, in the period before

TABLE 4

*Deviations of actual testing dates from the established centering-day in Herds I and II*

Herd	No. of testing periods	Average deviation (days)	
		Arithmetic	Algebraic
I .....	72	1.9	-1.7
II .....	42	9.7	+7.8

they begin production, at a higher level than that which is used (after the tenth testing period) to complete the 305 days of actual yield.

The significantly higher mean of the centered records compared to the mean of the actual milk yields in Herd I (table 3) is caused, in part at least, by the centering scheme's back-credit calculations. Part of this difference between means arises from the large number of records of "back-credit" cows, in which the rate of production used to calculate back-credit is higher than the actual rate of production during this back-credit period. It also arises from the "non-back-credit" records in those cases in which the tester's first visit comes near to the peak of the lactation curve.

The data for Herd II show that the average of the centered estimates is lower than the mean actual milk yields. The consistently late testing dates in this herd are undoubtedly the explanation for this lower mean. That is, the frequency of the "back-credit" cows is reduced about half, and the production estimates for the centering periods during the declining phase of the lactation curve are based on testing day yields taken near the end of each period; at this time the yields are lower than the average actual daily yields for those periods.

The repeatabilities (intra-cow correlations) for the two estimates of butterfat and milk production and for actual milk production are given in tables 2 and 5. There is not a significant difference between the repeatabilities of the simplified and centered estimates of butterfat production, nor are there any significant differences between these repeatabilities for the simplified, centered and actual milk records within herds.

TABLE 5

*Repeatabilities of simplified and centering methods and actual milk production records in Herds I and II*

Herd	Number of cows	Simplified	Centering	Actual
I .....	60	0.36	0.35	0.38
II .....	16	0.55	0.53	0.52

## SUMMARY AND CONCLUSIONS

A simplified scheme for computing 305-day milk and butterfat records has been described, and such records have been compared statistically with those estimates calculated by the D.H.I.A. centering method and with actual 305-day milk figures.

The high correlations found between simplified, centered and actual milk records and between simplified and centered butterfat estimates, and the close similarity of their intra-herd repeatability figures and mean yields for milk and butterfat production indicate no important differences between the simplified and centered schemes.

The simplified scheme would offer, however, the following advantages:

1. It would avoid most of the sources of those arithmetical errors which so often occur in the centered calculations.
2. It would facilitate the training of testing supervisors.
3. It would provide the tester with extra time so that more cows could be tested in one day, or more time could be devoted to conferences with the dairy farmer.
4. It would allow the dairy farmer to understand readily the method used in estimating production records, and to make the calculations himself if necessary.
5. It would save much time in the recording and checking of production data which are to be used for research purposes.

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# THE STRUCTURE AND PROPERTIES OF THE NATURAL FAT GLOBULE "MEMBRANE"\*

## A HISTORICAL REVIEW WITH EXPERIMENTS BEARING ON A PHYSICO-CHEMICAL EXPLANATION

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### HISTORICAL

Babcock (2) probably was the first American chemist to investigate emulsion character of milk and cream and to reject Ascherson's (1) haptogen membrane theory which had already prevailed for 45 years.

According to Babcock (2), in 1885 the fat globules were considered to be either (a) "particles of free fat, in the form of an emulsion with the serum of the milk," or (b) "surrounded by a thin membrane, and therefore cells filled with fat," or it was believed (c) "that the albuminous matter of the milk is attracted and in some way condensed upon their surface, forming what is called *haptogenic* membrane."

It is not clear why Babcock distinguished between the "cells filled with fat" and the haptogen membrane theory of Ascherson (1) (whom Babcock did not mention by name) since Ascherson's paper clearly considers capillary condensation of albumin and aggregation of an infinite number of small particles at fat surfaces to produce "haptogen" membrane as identical descriptions of the property of "hymenogeny" which he discovered. Furthermore, Ascherson not only postulated that the fat globules in milk are surrounded by a "haptogen" membrane but he claimed to have seen the membrane by microscopic observation, both in natural milk and in artificial emulsions of olive oil in dilute egg albumin solution.

Babcock disagreed with those who claimed to have seen the membrane both intact and after rupture as the "broken sacks" of the fat globules by suggesting a possible "lack of skill in the use of the microscope" as well as "influence of a preconceived opinion." It is obvious that Babcock himself favored the emulsion theory to account for the fat globules. An emulsion, he stated, is produced by dispersing liquid fat in an aqueous fluid having viscous properties whereby the fat particles are prevented from uniting again "by a thin film of liquid analogous to that which separates the bubbles of air in foam or soap suds." Babcock then pointed out the analogies between milk and artificial emulsions, (a) in microscopic appearance,

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(b) in creaming and the accentuation of the same by dilution with water, (c) on churning, especially the relation thereto and importance of temperature and melting point of fat, and (d) on their behavior when treated with ether. As a convincing argument Babcock pointed out that churns which had formerly been designed with the view of rupturing the laptogenic and other alleged membranes surrounding the fat globules were being replaced with those which would accentuate aggregation and coalescence of the fat globules into granules and of the granules into butter.

Babcock was soon led to modify his views somewhat regarding fat globule "membranes" due to his conclusion that traces (Babcock estimated 0.0002 per cent) of fibrin normally form in milk. In 1889 (3, 4) Babcock presented the evidence for the formation of "lacto-fibrin" and discussed the importance of this phenomenon at considerable length. While neither the evidence nor the alleged practical applications of the phenomenon are any longer valid, it is of interest that Babcock sought by means of his conclusions to explain the natural agglutination of the fat globules,<sup>1</sup> the relation thereto of gravity and centrifugal creaming and its influence on churning. Babcock believed that in cream the fibrin "clots have practically the same effect as would a true membrane covering the globules, and must be removed before the globules can unite in the form of butter." The acid of ripened cream was regarded as an effective solvent for this purpose. At the present time students who are being introduced to current theories in dairy chemistry are usually surprised to learn that while Babcock was one of the first to call attention to the natural fat globule clustering phenomenon, and later (5) showed its importance in explaining changes in the "consistency" (viscosity) of milk, he nevertheless regarded fat clustering as detrimental to the creaming and described methods for preventing it.

Current theories about the emulsions which occur naturally in biological material permit the acceptance of aggregates of oriented colloidal particles at the surface of milk fat globules to form "membranes" such as were not visualized by Babcock. Some of Ascherson's views, however, were remarkably prophetic of later discoveries.

The fact that milk plasma contains a number of proteins each capable of effective stabilization of fat emulsions has been the cause of much of the confusion in the literature as to whether cows' milk possesses a special fat emulsifying system. For a number of years I have employed the term membrane in quotation marks to describe this system. A major part of this literature, both old and more recent, may be found in the following publications (6, 16, 17b, 18, 20, 25, 30, 31).

Although Babcock (2) recognized that the milk emulsion is not destabilized by dilution with water, the Danish chemist Storch (24) was the first to

<sup>1</sup> The occurrence of a true agglutinin in milk which is adsorbed by the fat globules at low temperatures, causing their agglutination, is supported by the paper of Sharp and Krukovsky (*Jour. Dairy Sci.*, 22: 743-752, 1939) in which the older literature is reviewed.

apply this fact to the problem of isolating the natural emulsifying agents through the process now commonly called cream washing. Separator cream is diluted with distilled water at approximately body temperature (Storch used both water and sucrose solution) to give a fat content similar to whole milk and the diluted cream re-separated. If one starts with fresh cream from the freshest possible milk this process may be repeated until the tests of the washings for milk plasma constituents are essentially negative without impairing the stability of the emulsion. Surface and interfacial adsorption equilibria are, of course, sensitive to temperature changes. If the cream washing operation is conducted at animal temperature it is reasonable to conclude that the fat globules of such washed cream are "coated" by the emulsifying agents present when the milk is secreted together with other substances whose presence could be explained by chemical or physical affinity with the fat or its emulsifying agents. With these hypotheses as a background, it becomes largely a chemical problem to isolate and identify the natural "membrane" components.

Storch's (24) experiments pointed the way to an important aid in the isolation of the "membrane," which I and my associates (16) developed and employed extensively in our later studies (17a, 18, 20, 25, 30, 31), namely, the release of a large part of the protective agents into the buttermilk during the churning of washed cream whereby both the free buttermilk and that released by melting the butter become important source materials for chemical studies. Indeed, not only was it found (20, 29) that the emulsion properties and churnability of artificial emulsions of milk fat in the various colloidal sols from milk plasma are strikingly different from those of washed natural cream but also that the "membrane" materials isolated from the washed artificial creams are also chemically distinct from the natural "membrane" substances.

Only a relatively brief account can be given of the discovery of the specific components of the natural "membrane." Storch (11) first postulated a specific protein but its supposed mucoid nature was not substantiated. The protein is salted out of aqueous sols like a globulin (16) but does not require electrolytes for dispersion (18). As isolated by Hattori (10), by Samuelsson (16) and by Wiese (18), by Rimpila (20), and by Tarassuk (17b), and by Schwarz and Fischer (22), the protein is characterized by a N content several per cent lower than that of any other milk protein, a fact not yet explained either on the basis of known amino acid composition or identified prosthetic groups (30, 10, 22). The biological specificity of the protein was established by Lewis (15).

Dornic and Daire (8) postulated that the higher lecithin content of buttermilk than of whole milk arose from the release of lecithin from Storch's fat globule "membrane" but Samuelsson (16) first supplied direct evidence in support of this hypothesis by isolating phospholipides from washed cream

buttermilk. Wiese (18) later identified lecithin, cephalin and sphingomyelin-like phosphatides in the natural "membrane" material. Thus, the specific emulsion stabilizing agent of cow's milk was finally established as a protein-phospholipide complex. Its aqueous sol was found (18) to have an isoelectric point at pH 3.9-4.0. The importance of the phospholipides in the hydrophilic properties of the membrane has been emphasized by Pyenson and Dahle (19). Sandelin (21) believes that lecithin is the more important component of the membrane in explaining the stability of the milk and cream emulsion.

A third major lipid component of the isolated natural "membrane" was encountered by Wiese (18) and Rimpila (20) in the form of a neutral, high melting glyceride, the significance of which is still obscure.

The more important components of the natural "membrane" present in minor quantity are enzymes and heavy metals. Toyama (26) first showed that the crude "membrane" serves as a satisfactory concentrate of xanthine oxidase, thus confirming the view of Wieland and Macrae (29) that cows' milk dehydrogenase is closely associated with the fat globules. Sharp (23) states that about one-half of the xanthine oxidase may be removed from the fat globules by washing. He also reports that milk contains about 70 milligrams of the enzyme per 100 grams of fat. This would constitute 12-15 per cent of the protein of the membrane, based on Rimpila's (20) data regarding the protein:fat relationship of washed cream. Xanthine oxidase being a riboflavin-protein compound accounts for the fact that both raw sweet cream buttermilk and the buttermilk from churning washed cream have a brownish-yellow color. Kay and Graham (13) first demonstrated that phosphatase is concentrated on the fat surfaces of milk. Rimpila (20) found that 50 per cent of the Kay-Graham phosphatase of fresh cream remains after six washings, each with four volumes of water. Davies (7) found that the "membrane" protein readily combines with copper and iron which dissolves in milk and cream in milk processing plants, thus causing a concentration of these metals on the surface of the fat globules. The probable importance of metallic ions, especially of copper ions that might arise from these compounds, in contributing to the oxidative deterioration of cream and butter, may readily be conjectured.

#### EXPERIMENTAL

The character of the specific components of the natural fat globule "membrane" of cows' milk so far identified raises both physiological and physico-chemical questions regarding their origin. The supposition that they are artifacts of the cream washing procedure is not rational. Physiological explanations of their origin are still in the realm of speculation but it is difficult to ignore the thought that there is some intimate relationship between them and the synthesis and secretion of milk fat. The physico-

chemical aspects of the question are capable of experimental approach. Milk plasma contains colloidal systems which are excellent emulsion stabilizers. If it could be determined that the specific components of the natural membrane are preferentially adsorbed by a milk fat surface because of normal differences in their interfacial tension reducing ability as compared with the plasma colloidal systems, the origin of the natural "membrane" would have a physico-chemical explanation which could be regarded as at least plausible.

Dr. M. E. Powell carried out an extensive study of this question in my laboratory during 1932-34.<sup>2</sup> None of the results have heretofore been pub-

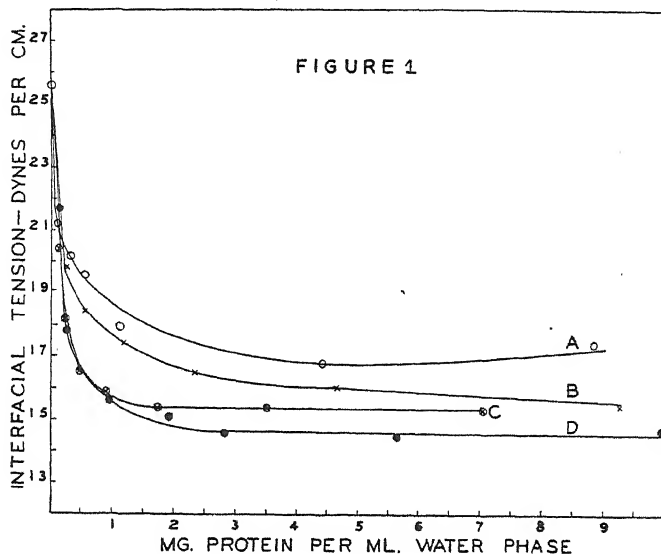


FIG. 1. Comparative tension reducing ability at water-butter oil interface of proteins (1) in lactalbumin sol A; (2) mixed milk serum protein sol B; (3) milk plasma sol C; and (4) calcium phosphocaseinate sol D, pH 6.7. Readings at 40° C.

lished. Using the drop weight method of Harkins and Brown (9) the interfacial tension reducing ability of different concentrations of the various colloidal systems of milk, including the natural "membrane" system, was determined at a butterfat-water interface at 40° C. This made it possible to determine at what concentration of protein a minimum interfacial tension was attainable for each system and gave a comparison both of the absolute ability of each material to reduce the interfacial tension of the butterfat-water interface and of the relative effectiveness of each to produce its own minimum tension.

In this work we observed for the first time that freshly washed fresh cream is sufficiently stable to withstand homogenization at 3000 lbs. pres-

<sup>2</sup> This work was supported by a grant from the Rockefeller Foundation.

sure at 105° C. This suggests that the materials remaining at the fat surfaces of washed cream represent either multimolecular layers or monomolecular complexes capable of considerable distensibility without impairment of the stability of the emulsion. The capacity of the resulting interface to adsorb the various colloidal capillary active systems of milk was examined experimentally in interfacial tension studies.

Figures 1, 2, 3, 4 and 5 present graphically some of the results of the various interfacial tension measurements.

Figure 1 shows the relative interfacial tension reducing ability of (a) lactalbumin sol (whey dialyzed against distilled water), (b) milk serum proteins sol (whey dialyzed with addition of NaCl), (c) milk plasma (skim

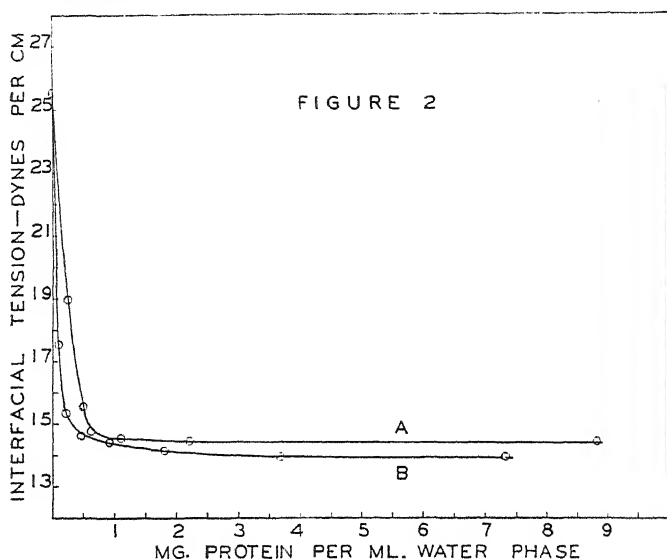


FIG. 2. Comparative tension reducing ability at water-butter oil interface of proteins in (1) milk plasma sol A; (2) sweet cream buttermilk B, from the same milk. Readings at 40° C.

milk) and (d) calcium phosphocaseinate sol (pH 6.7), each per unit of protein, their effectiveness being in the order named. Figure 2 shows the definitely greater effectiveness of sweet cream buttermilk over milk plasma proteins in reducing interfacial tension, both products being from the same original milk.

Figure 3 shows the markedly greater effectiveness, on the protein basis, of buttermilks from the melted butters of washed creams (curves A and B) than of the free buttermilks of the same washed creams (curves C and D) in reducing interfacial tension. These results seem to support the recent findings of Maïmistova (14) that the capillary activity of the fat globule "membrane" is conditioned by its phospholipide compounds. We have, in



our laboratory, unpublished evidence that relatively greater proportions of phospholipide remain in the butter churned from washed cream than is liberated in the free buttermilk. The findings of Sandelin (21) also support this.

Figure 4 shows the effects on interfacial tension between butterfat and homogenized washed cream of additions to the latter of two samples of milk plasma (skim milk), curves A and B, a calcium phosphocaseinate sol, curve C, and a concentrate of free buttermilk from an unhomogenized portion of the same washed cream which had been homogenized, curve D. The rise

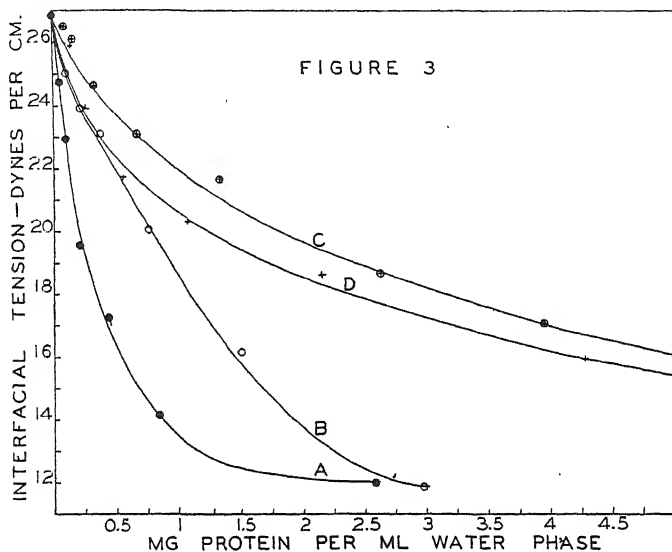


FIG. 3. Comparative tension reducing ability at water-butter oil interface of protein in (1) buttermilks from melted butter of washed creams A and B; (2) free buttermilks C and D, sols A and C being from the same washed cream, likewise B and D. Readings at 40° C.

in interfacial tension after the minimum had been reached in curves C and D suggests regions of concentration of the materials where less adsorption of capillary active colloids on the homogenized fat globule surfaces occurred. Why this should be so is not clear. Attention is called to the fact that figure 4 requires a different interpretation than figures 1, 2 and 3 since we are probably dealing here with colloids which remain free to reduce the tension between the butter oil and the water phase of the homogenized washed cream after the fat surfaces of the latter have attracted such material as can be adsorbed.

Figure 5 shows further evidence in support of the conclusions drawn from figure 3, that the phospholipide-protein complex retained by the butter of washed cream is more effective, on the protein basis, than the free butter-

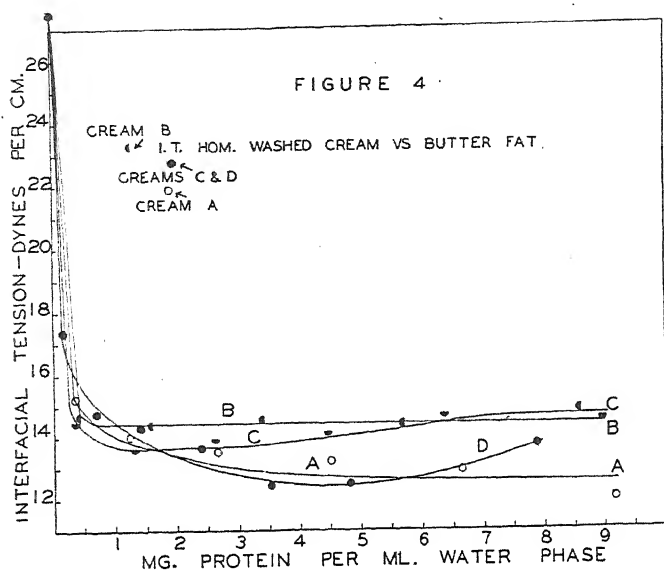


FIG. 4. Comparative tension reducing ability at homogenized washed cream-butter oil interface of proteins in (1) milk plasmas A and B; (2) a calcium phosphocaseinate sol C; and (3) a concentrate of free buttermilk D from an unhomogenized portion of the same washed cream which had been homogenized to furnish the interface. Readings at 40° C.

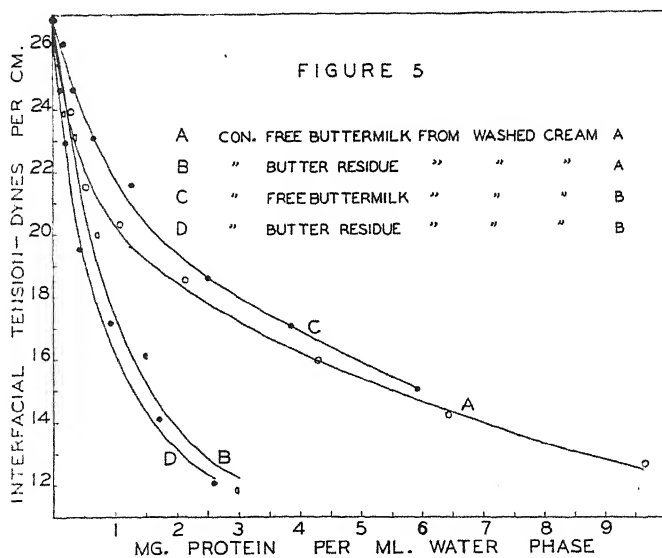


FIG. 5. Comparative tension reducing ability at water-butter oil interface of proteins in (1) concentrated free buttermilk A and concentrated aqueous phase of butter B, both from the same washed cream A; (2) concentrated free buttermilk C and concentrated aqueous phase of butter D, both from same washed cream B. Readings at 40° C.

milk from the same washed cream in reducing the tension at a melted butter-fat-water interface.

*General importance of "membrane" in dairy technology.* The demonstrated fact that the fat phase of cows' milk is stabilized by a complex (or complexes) of the lecitho-protein type, involving a specific protein and having associated with it concentrations of redox and phosphatase enzymes, suggests numerous problems involving their probable importance in dairy technology. Both Wiese (18) and Rimpila (20) found certain variations in composition of the natural "membrane" which are no doubt of considerable significance. The relation of these facts to various theories of churning has been discussed by Palmer and Wiese (18). It cannot be questioned that there is considerable release of natural "membrane" during the churning and theories supposing the necessity of foaming and of coagulation of the "membrane" protein are made untenable.

Thurston and Barnhart (27) found important relations to exist between "richness" of flavor in milk products and the phospholipide fractions of the "membrane." Thurston and associates (28) presented evidence supporting their belief that certain off flavors of milk, particularly oxidized flavor, are associated primarily with chemical deterioration of the lecithin of the fat globule "membrane" rather than with oxidation of the butterfat itself. Jack and Dahle (11) have presented evidence suggesting the probability of a double layer membrane on the surface of the fat globules, the outer layer of which must be removed in order to secure centrifuged cream of highest fat content. That the natural "membrane" material released during churning may explain, in part at least, the low curd tension of sweet cream buttermilk is indicated by experiments carried out in the author's laboratory (17a). Many of the normal properties of natural cream have been found to require the natural "membrane." This is true for centrifugal cream separation, gravity creaming and churning (30) and for desirable whipping properties of ice cream mixes (12). For the latter the significant aspect is the protein-phospholipide complex which is thus capable of being imitated by other natural complexes of this type, *e.g.*, by that in egg yolk.

#### SUMMARY

The fat globules in cows' milk are wholly or partially surrounded by a special group of substances whose origin may be due, in part, to their greater capillary activity. The other surface active substances occurring in major concentration in milk plasma evidently constitute the outer layers of the fat globule surfaces if indeed they are normally concentrated there at all. The latter are readily removed when cream is washed by dilution with water. Experimental work in the author's laboratory and by numerous other workers cited, has pointed to the importance of the natural "membrane" of the fat globules in creaming, churning, milk flavor (both normal and oxidized),

decreased curd tension of natural sweet cream buttermilk, and in determining the desirable whipping qualities of ice cream mixes.

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## IMPROVING THE QUALITY OF SWISS CHEESE BY CLARIFICATION OF THE MILK<sup>1</sup>

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It is commonly recognized that good quality in the milk is an important factor in the production of high quality cheese. For making Swiss cheese the suitability of any milk appears to be enhanced by the agitation or centrifugal treatment given milk by clarification. Orla-Jensen (20) was the first to observe that centrifuging milk resulted in improvement in Emmentaler (Swiss) cheese by the formation of fewer and larger eyes, and that a similar effect resulted when the cheese milk was filtered and likewise when it was agitated more than usual while being transported for a long distance. This process for improving Swiss cheese was first described in this country by Matheson in a preliminary report (16) and in a public service patent (17) from these laboratories. Its use has resulted in such marked improvement in eye formation and in the quality of the cheese generally that it has been adopted in practically all Swiss cheese factories in this country. The most obvious effect on the quality of the cheese is an increase in the size of the eyes and a decrease in their number, this resulting in a distinct improvement in grade and market value. The effects on the cheese appear to be caused by alterations in the composition of the milk and in the physical condition of the constituents of the milk, both of which factors result in conditions conducive to bacterial action of a type which favors the development of the proper texture in the curd and desirable eye formation.

Previous to 1924 little was known as to the mode of action of clarification in improving the quality of the cheese. It was thought to come about through "removal of dirt or other cellular elements from the milk" (17), and it was mentioned also (16) that the process "breaks up the clusters of fat globules." Orla-Jensen (20) believed that the improvement resulted from the effects of agitation in distributing uniformly gas-forming organisms, especially those associated with particles of foreign material. More recently, Guittonneau and his associates (6) indicated that particles of for-

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<sup>1</sup> The cheesemaking phases of the investigations were directed by the late Kenneth J. Matheson, who was largely responsible for improvement in quality of domestic Swiss cheese by clarification. The introduction in the factories of the clarifying process, and also of the pure culture method, was accomplished largely by other former employees, including Sumner A. Hall, Robert E. Hardell, James A. Boyer, Robert R. Farrar, and Fred Feutz, and by H. R. Lockry of this Division.

<sup>2</sup> Deceased April 24, 1940.

eign material, when deposited in the cheese, act as centers of proliferation of undesirable gas-forming organisms, and that the improvement in eye formation results from the removal of large numbers of the organisms that occur as contaminants in and on the heavier particles of sediment.

Some of the favorable effects reported as due to clarification of milk on cheese include increased firmness of body of Swiss cheese (16), improved body (3, 5, 34) and flavor (3, 5, 22, 34) of Cheddar cheese and the prevention of gassiness, pinholes, and sponginess (1) in the curd of Cheddar cheese.

Reports of the effect of clarification on the milk in the removal of extraneous matter and cellular material have shown that the clarifier removes practically all visible sediment (10, 13, 19), that a portion of the fibrin (common in mastitis milk) is removed (13), that from about two-fifths to about two-thirds of the leucocytes and body cells are removed (4, 7, 10) and many of those remaining in the milk are fragmented (15), and that the proportion of cells removed increases as the temperature of clarification is increased (10). Some investigations have shown that the larger types of organisms are removed in greater proportion than the smaller ones (13), and also that, when the rate of flow of milk through the bowl is reduced, there is some selective removal of different types of bacteria, causing actual decreases in plate counts (34). On the other hand, the numbers of streptococci in the milk are not reduced significantly (28) and investigators (4, 7, 10, 13, 34) have agreed that clarification results in an increase in the total number of bacteria in the milk as determined by the plate count, due to breaking up of chains and clumps of bacteria. This change in the bacterial flora by clarification does not improve the keeping quality of the milk, since it has been shown that the methylene blue reduction time is often decreased (10, 28) and that there is a slight increase in the rate at which acidity develops (7, 19, 28).

It has been stated that clarification results in a reduction in size and an increase in the number of fat globules (19, 4) in milk and breaks up fat clumps (4). It is well known that, at temperatures used commonly, the process causes a decrease in the rate of creaming and a decrease in the volume of gravity cream (4, 7, 10, 14, 19, 33); at relatively high temperatures it causes an additional decrease in the volume of gravity cream (4, 10, 33). A reduction in the volume of gravity cream is produced also by pumping milk with a centrifugal pump (33), and by mechanical agitation (30). The latter treatment was found to break up clusters of fat globules. Agitation is believed to alter the surface characteristics and adsorption on the fat globules (11), with which the creaming property is associated (21); the fat-clustering agent, agglutinin, believed to be present in milk, is thought to be removed to some extent by agitation and centrifugal force (8); and the agglutinating material, when adsorbed on the surfaces of solid fat globules, can be released rather readily (27) by such a mild treatment as raising the



temperature to above that of the melting point of the fat. Further indication that clarification of milk alters the nature of the fat is provided by evidence that, in the manufacture of dried milk, clarification of the milk increases the resistance of the fat to oxidative decomposition and improves the keeping quality (9).

The alteration in the gaseous content of milk resulting from the centrifugal agitation in the clarifying process may be considered as another desirable factor attributable to clarification in the manufacture of cheese. Babcock (1) showed that the centrifuging of milk tended to prevent gassiness in Cheddar cheese curd and he believed this effect was due to aeration, which presumably alters conditions for bacterial growth and inactivates certain types of gas-forming bacteria. Marshall (12) found that agitation of milk promoted an interchange of gases, thus increasing the ratio of oxygen to carbon dioxide, and that such an interchange inhibited undesirable fermentations. Results of Matheson (16) showed that the addition of ozone or of oxygen to milk inhibited the gassy fermentation produced by a vigorous, gas-producing, spore-forming anaerobe in Swiss cheese, without apparent injurious effect on eye formation or on development of the lactobacilli in the cheese.

It is obvious that clarification effects removal of the extraneous matter and also alters the physical properties of some of the constituents of milk. The evidence available indicates also that the changes produced by clarification result in conditions more favorable for the growth of the types of organisms that are useful in the cheesemaking process. From the wide variations that appear in the quality of cheese made commercially from clarified milk, however, it appears that its effects are more pronounced in milks possessing certain abnormalities, and that in some cases the milk is not clarified properly. This paper is a report of experiments designed to contribute additional information on the effects of clarification on the properties of milk; to study the effects of certain related treatments of milk which influence the quality of Swiss cheese; and to verify in a quantitative way the effects of clarification on the quality of the cheese. During part of this investigation there was available a supply of milk selected from cows having mastitis, and the results of clarification studies on this milk are included because of the unusually pronounced improvement that occurred when abnormal milk of this type was clarified.

#### METHODS

In efforts to account for variations in the quality of the cheese, those properties of milk which appeared to be of possible significance were determined in samples of the mixed milk taken from the cheese kettles. Counts of clusters of fat globules and of numbers of globules per cluster were made, at a temperature of 25° to 26° C., by means of a microscope in milk samples

diluted 1:24 in an aqueous solution containing 1.5 parts of gelatin and 1 part of phenol per 100 parts. The phenol was used as a preservative in the gelatin solution after it was determined that its presence in the test did not affect the tendency of the fat globules to form clusters. Pairs of samples were placed in a calibrated Levy counting chamber (hemacytometer) with double ruling and allowed to remain for one to one and one-fourth hours for clustering to occur before counts were made. At least 50 fields per sample were examined. Groups containing 10 or more globules together were counted as clusters. For measuring sizes of fat globules an ordinary cover glass was used on the counting chamber and measurements were begun at once without allowing time for clustering to occur. A calibrated ocular micrometer disc, and also an ocular disc with circle and cross lines, were used in measuring and counting.

The creaming ability of milk was measured in samples held for 24 hours in 100-ml. graduated cylinders of uniform height immersed in a water bath at 3° to 5° C. (graduated cylinder method).

The creaming ability of milk was measured also by a centrifugal procedure designed to shorten the time required for the completion of creaming and designated as the cream test bottle method. Large-bodied, 50 per cent, nine-gram cream test bottles were selected which, when filled to the 50 per cent mark, contained not less than 55.2 ml. Two 17.6-ml. samples of milk, a total of 35.2 ml., were pipetted into each bottle and 20 ml. of water was added. The sample was mixed thoroughly and then centrifuged for one hour at room temperature in a Babcock centrifuge, after which the volume of cream was read with the aid of a light placed behind the bottle. Since each whole percentage division represents a volume of 0.1 ml., the percentage of cream is calculated by multiplying the whole percentage unit volume of cream by 0.1, dividing by the volume of milk used (35.2 ml.), and multiplying by 100.

Methods described in an earlier publication (25) were used for determining rennet curd tension, rennet coagulation time, and stability to alcohol. Determinations of pH values were made by the quinhydrone electrode method described earlier (23). Amounts of oxygen and of other gases were determined by means of the Van Slyke (31) manometric blood gas apparatus. The numbers of organisms in the kettle milks were determined by the standard plating method in tomato-milk powder agar, and the numbers in cheese were determined microscopically (2). The counts of mastitis streptococci were made in Edwards' blood agar medium.

In the cheese experiments, cheese was made in pairs from two weighed portions of the same lot of mixed milk, and each test cheese was made in the same manner as the control except for the experimental variations described. The clarifier used was of the old style and studies with the new no-foam clarifier were not made. The usual clarifying temperature was 28–30° C.

The internal diameter of the clarifier bowl was 204 mm. (8 inches), the capacity 4,000 pounds per hour, and the rated speed 7085 rpm. Each experimental cheese weighed 55–62 pounds when removed from the press. Curing conditions and details of grading were the same as those described earlier (24).

The starter culture used in the earlier experiments was 39a (now identified as *Lactobacillus lactis*). In later experiments, either B<sub>2</sub> (a culture of *Lactobacillus bulgaricus*) or Ga (a mixed culture of a lactobacillus with a mycoderma) was used. While the Ga culture has been used extensively in the manufacture of Swiss cheese in this country for a number of years, studies of the lactobacillus in this culture have not as yet shown it to be identical with any species of lactobacillus described in the literature. The later experiments included the use of *Streptococcus thermophilus* cultures in addition to the lactobacillus cultures referred to above.

A penetrometer was used for determining softness of body of cured cheese. It consisted of a cylindrical plunger one-eighth inch in diameter, with a flat end, mounted on a frame, surmounted by a 200-gram weight, and connected to a needle which moved on a dial graduated to show the movement of the plunger in hundredths of a centimeter. The distance that the plunger sank into a small block of cheese in 15 seconds was recorded, and the average of five determinations was taken as the penetrometer reading. Determinations were made at a constant temperature of 18° C., and also usually at two different temperatures so that changes in softness with temperature could be plotted.

#### EFFECTS ON PROPERTIES OF MILK

The average volume percentages of cream obtained on 12 pairs of samples of milk were as follows: Graduated cylinder method—clarified milk, 10.2; unclarified milk, 10.9; cream test bottle method—clarified milk, 9.8; unclarified milk, 10.9.

Measurements of the sizes of the fat globules determined microscopically in a large number of samples showed that the decrease in creaming could not be accounted for on the basis of a diminution of sizes of the globules. There was a slight increase following clarification in numbers of those more than 4  $\mu$  in diameter and a more evident increase of those more than 6  $\mu$  in diameter. Some relatively large globules (larger than 10  $\mu$ ) were found much more frequently in clarified than in unclarified milk, and some of the largest globules were found to have apparently coalesced during clarification. The resulting clumps were in some cases non-spherical in shape, and this effect was greater in milks clarified at 32° C. than in those clarified at 21° C.

It was found that the tendency of the fat globules to aggregate in clusters during creaming was reduced greatly by clarification. Data show-

ing the effect of clarification on clustering and the effects of different modifications of the clarifying process on the numbers of clusters are shown in table 1. Counts in a large number of samples showed also an average reduction of about 20 per cent in the numbers of globules per cluster following clarification.

When cream that had been allowed to rise by gravity was clarified and remixed with the original skim milk it was found that the tendency of the fat to aggregate in the milk was reduced. A similar effect on clustering was produced when the gravity cream was agitated for 10 minutes at 40° C. and then remixed with the original skim milk.

TABLE 1

*Effects of clarification and of other treatments of milk on average numbers of fat clusters in milk and on average extent of over-setting defect in Swiss cheese*

Treatment of milk	Pairs of samples	Fat clusters per 0.01 cu. mm. milk		Eyes per cut surface of cheese	
		Number	Decrease in number	Number	Decrease in number
	<i>number</i>		<i>per cent</i>		<i>per cent</i>
Not clarified .....	30	186	...	106	...
Clarified .....	...	73	61	54	49
Clarifier speed 3500 rpm. ....	25	156	...	77	...
Clarifier speed 7000 rpm. ....	...	72	54	59	23
Clarified at 21° C. ....	26	134	...	73	...
Clarified at 32° C. ....	...	86	36	64	12
Gravity cream not clarified	16	173	...	98	...
Gravity cream clarified .....	...	92	47	65	34
Gravity cream untreated .....	10	172	...	118	...
Gravity cream heated and agitated .....	...	66	62	103	13

Results of the alcohol test showed that stability of milk proteins to alcohol is decreased slightly by clarification, indicating that the process alters the properties of the casein slightly.

The rennet coagulation time of milk was found to be unchanged following clarification. The rennet curd tension was not altered significantly. The pH value was usually unchanged, but in some instances was reduced slightly. The methylene blue reduction time at 37° C. was usually not changed materially but in some instances was decreased slightly.

In experiments in which 18 lots of mastitis milk were made into cheese (table 3), the average of the numbers of leucocytes was decreased by clarification from 2.4 millions to 0.7 million per milliliter, or about 70 per cent. The numbers of streptococci and of other bacteria were decreased also, but with less consistency and to a less extent. The percentage reduction in numbers of leucocytes tended to be greatest in those milks containing the

largest numbers. The reduction varied directly with the speed of the clarifier bowl and with the temperature at which the milk was clarified, and inversely with the rate of flow of the milk through the clarifier.

Effects of clarification and of the addition of oxygen and of carbon dioxide upon the gas content of milk are shown in table 2. Clarification increased the oxygen content, decreased the carbon dioxide content, and decreased the total gas content. It will be noted that the interchange of gases resulting from clarification is in the same direction as that produced by the artificial introduction of oxygen, which has been shown by Marshall

TABLE 2

*Effects of clarification, and of treatment with gases, on the percentages of gases in milk*

Treatment of milk	Pairs of samples	Duration of gas treatment	Average amounts of gases in milk			
			Oxygen	Carbon dioxide	Nitrogen and residual gases	Total
	<i>number</i>	<i>minutes</i>	<i>volumes per cent</i>	<i>volumes per cent</i>	<i>volumes per cent</i>	<i>volumes per cent</i>
Clarified .....	14	.....	0.60	1.75	1.20	3.55
Not clarified .....	.....	.....	0.55	2.10	1.20	3.85
Oxygen added* .....	13	10	0.69	1.84	1.29	3.82
Normal .....	.....	.....	0.59	1.93	1.37	3.89
Carbon dioxide added* .....	7	5	0.52	3.58	1.22	5.32
Normal .....	.....	.....	0.58	1.84	1.24	3.66

\* Gas bubbled through small openings in a perforated coil, at rate of 25 liters per minute, into 700 lb. milk in cheese kettle; temperature of milk, 28-30° C.; samples analyzed immediately after treatment.

(12) and by us (16, 18) to have a favorable influence on the activities of desirable types of organisms in milk.

Direct counts of Swiss cheese starter organisms made microscopically on five pairs of inoculated samples of milk that were subjected to the same temperature conditions existing in the cheesemaking process showed that the growth of the lactobacilli began considerably earlier in clarified than in unclarified milk and that the growth of both the lactobacilli and the streptococci progressed more rapidly in the former than in the latter. It was found also that, in milk samples held at 30° C., clarification caused the oxidation-reduction potential to decrease more rapidly.

#### EFFECTS ON QUALITY AND PROPERTIES OF CHEESE

Data for 369 pairs of cheese, showing the improvement of quality resulting from clarification and also the effects of various modifications of the clarifying process, are presented in table 3. In the cheese made from normal milk by the usual clarifying process (group 1), practically all of the im-

TABLE 3

*Improvement in quality of Swiss cheese by clarification of milk; and effects of modifications of the clarifying process. (Averages for 60-lb. cheese cured 2½ to 3½ months)*

Treatment of milk	No. of pairs	Scores of cheese*			Proportion of cheese in each grade			
		Eyes	Body and texture	Total	No. 1	Special	No. 2	Grinder
		<i>points</i>	<i>points</i>	<i>points</i>	%	%	%	%
1) Normal milk, clarified .....	112	25.4	23.0	75.2	49.1	34.8	9.8	6.3
Normal milk, not clarified .....	.....	18.7	23.8	69.0	11.6	37.5	40.2	10.7
2) Mastitis milk, clarified .....	18	22.4	24.0	73.5	22.2	61.1	16.7	.....
Mastitis milk, not clarified .....	.....	12.2	23.2	61.2	5.6	11.1	22.2	61.1
3) Clarified .....	4	25.2	23.0	74.7	25.0	50.0	25.0	.....
Separated .....	.....	23.0	21.5	71.2	.....	50.0	25.0	25.0
4) Clarifier speed—7000 rpm. ....	65	22.0	23.0	72.0	30.8	32.3	24.6	12.3
Clarifier speed—3500 rpm. ....	.....	21.0	23.5	70.0	24.6	24.6	36.9	13.9
5) Clarified at 32° C. ....	63	23.8	22.7	73.2	44.5	23.8	23.8	7.9
Clarified at 21° C. ....	.....	21.6	22.4	70.5	28.6	22.2	34.9	14.3
6) Normal rate of flow of milk through clarifier .....	16	24.2	22.4	73.6	31.3	37.5	25.0	6.2
Slow flow, half normal rate .....	.....	25.2	23.8	75.6	50.0	31.3	12.5	6.2
7) Sediment from bowl returned to clarified milk .....	54	23.1	24.0	73.4	44.4	31.4	13.0	11.2
Milk clarified, sediment not returned to milk .....	.....	23.2	22.0	71.7	29.6	29.6	29.6	11.2
8) 90-lb. gravity cream not clarified, 600-lb. gravity skim-milk clarified .....	36	17.6	23.1	67.7	5.5	36.1	27.8	30.6
90-lb. gravity skim-milk not clarified, rest of milk clarified .....	.....	22.3	22.7	71.6	38.9	19.4	27.8	13.9
9) 90-lb. gravity cream clarified, 600-lb. gravity skimmilk not clarified .....	1	23.0	25.0	78.0	100.0	.....	.....	.....
90-lb. gravity skim-milk clarified, cream and rest of milk not clarified .....	.....	18.0	24.0	72.0	.....	100.0	.....	.....

\* Perfect score in points, according to scorecard used: eyes, 40; body and texture, 30; flavor, 20; and appearance, 10.

provement was in eye formation, *i.e.*, the eyes were larger, less numerous (less of the "oversetting" defect), shinier, and more regular and uniform in shape and distribution. There was a slight but uniform tendency toward more of the glaesler (curd-splitting) defect, and also more firmness of body, in the clarified than in the unclarified milk cheese. The former were generally slightly lighter in color and tended to rise somewhat more slowly in curing in the warm room. There were scarcely ever any detectable differences in flavor. Effects of different variations of the clarifying process upon the extent of the oversetting defect in the cheese are shown in table 1. The results show a striking correlation between the reduction in the fat clustering tendency in the milk, as affected by clarification, and the reduction in the oversetting defect in the cheese.

The greatest improvement resulting from clarification occurred when mastitis milk was used (group 2). Cheese made from this milk, without clarification, was wholly or partially pin-eyed (either pressler or nissler) in 15 instances out of 18. There were no pin-eyed cheese among those made from clarified milk. The unclarified, mastitis milk cheese was very soft or weak in body, relatively high in moisture content, and usually slightly inferior in flavor. Clarification caused an increase in firmness of body and a decrease in moisture content—factors which improve the quality of soft-bodied cheese.

Statistical tabulations of data for 110 wheels made from milks in which the leucocyte counts varied between 250,000 and 4,000,000 per milliliter showed a definite relationship between the numbers of leucocytes in the milks and the incidence and extent of the oversetting defect in the cheese. There was a strong tendency for high leucocyte counts in milk to be associated with "slow-working" or so-called "dead" milk in the kettle, with relatively slow development of acidity in cheese on the press, and with weakness of body in the cheese. These conditions are indicative of retardation of activity of starters and insufficient drainage of cheese on the press. They were improved markedly by clarification.

The use of the separator bowl did not produce as much improvement as the use of the clarifier bowl (group 3). In four experiments in which the milk was clarified twice, no additional improvement resulted from the second clarification. The improvement in quality was diminished when the speed of the bowl was decreased (group 4) and also when the milk was clarified at a relatively low temperature (group 5). The quality was improved when, with the bowl running at full speed, the rate of flow of milk through the bowl was diminished one-half (group 6). Results with the clarifier indicated that the improvement was generally proportional to the agitation or force to which the milk was subjected.

It is commonly believed that the improvement resulting from clarification is caused largely by the removal of extraneous matter or visible dirt.

However, the average quality of cheese made from clarified milk was found to be improved slightly, with respect only to the body, when the sediment from the bowl was returned to the clarified milk and mixed in thoroughly (group 7). Moreover, filtering the milk through cotton (12 pairs of cheese, unclarified milk) did not improve the quality of cheese to more than a very slight extent. Addition of the sediment did, however, cause a rather marked softening of the body of the cheese, which improved the body in this case because the cheese was ordinarily somewhat too firm; the average penetrometer reading on the cheese from clarified milk plus bowl sediment was 70; that on the normal controls, 45. The cheese to which the bowl sediment was added was also slightly more yellow and slightly less subject to the glaesler defect. The results indicated that improvement in eye formation cannot be ascribed to any great extent to the removal of visible sediment. It may be caused at least partially, however, by a change in the dispersion of the particles and by the effects of the process in breaking up bacterial clusters and distributing organisms more thoroughly in the milk, as Orla-Jensen (20) suggested.

Results of cheesemaking experiments with gravity cream (groups 8 and 9) indicated that effects of clarification on the fat in milk are apparently an important factor in improving the quality of cheese. When unclarified gravity cream (allowed to rise for 16 hours at 8–10° C.) was returned to the clarified milk (group 8), the quality of the cheese was so reduced as to make it comparable with that of cheese made from unclarified milk. When the gravity cream was clarified and then returned to unclarified gravity skim milk (group 9), improvement resulted which was comparable with that produced when all the milk was clarified. In further experiments made to determine effects of agitation on the fat, the gravity cream was warmed to 40° C., agitated vigorously for 10 minutes, and then re-mixed with the unclarified, gravity skim milk. This treatment resulted consistently in improvement in eye formation.

Prompted by results of Marshall (12) and of these laboratories (16, 18), referred to earlier, experiments were conducted to determine the effects of addition of oxygen and of carbon dioxide to milk. In 13 pairs of cheese the addition of oxygen (amounts shown in table 2) resulted in an average increase of six points in the scores of the test cheese. Twelve pairs were made with gaseous oxygen added to unclarified milk in the test kettle and with clarified milk in the control kettle. The average of the scores of the oxygen-treated, unclarified milk cheese was very nearly as high as that of the clarified milk cheese. Seven pairs were made from clarified milk with gaseous carbon dioxide added to the milk for the test cheese. The addition of carbon dioxide resulted in an average decrease of 10 points in the scores of the test cheese.

Results of counts of starter bacteria determined microscopically in samples taken from five pairs of cheese showed that the streptococci and



the lactobacilli, particularly the latter, multiplied more rapidly in cheese made from clarified than in that made from unclarified milk. A pronounced decrease in numbers of lactobacilli began after the cheese was one day old, and this decrease was more rapid in the unclarified-milk than in the clarified-milk cheese. The numbers of streptococci diminished somewhat later and more slowly than the numbers of lactobacilli, and they also diminished more rapidly in the unclarified-milk cheese. At three hours after dipping the average pH value in 112 pairs of cheese was 0.03 lower, and at eight hours 0.10 lower, in cheese made from clarified milk than in that made from unclarified milk. In nearly every instance cheese made from clarified milk contained less lactose when one day old than that made from unclarified milk.

There was a consistent increase in firmness of body of cheese as a result of clarification. Averages of penetrometer readings at 18° C. for 70 pairs

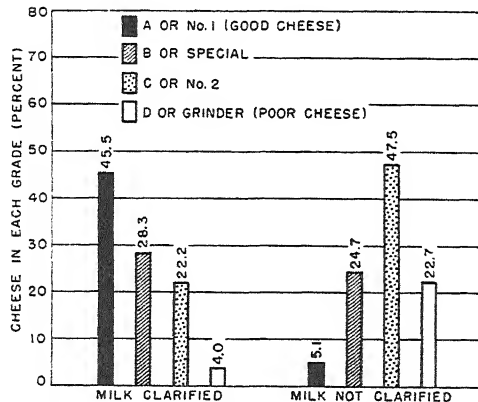


FIG. 1. Relationship between clarification of milk and quality of Swiss cheese (195 pairs of laboratory cheese).

of cured cheese made from normal milk were as follows: clarified, 35.1; unclarified, 55.0. Averages for 18 pairs made from mastitis milk were: clarified, 41.6; unclarified, 67.3. In many instances penetrometer readings were made at two or more temperatures and changes in softness were plotted against temperatures of readings. It was found that the clarified-milk cheese tended to soften less than the unclarified-milk cheese when the temperature was increased.

The glaesler (curd-splitting) defect occurred more commonly in cheese made from clarified than in that made from unclarified milk; the average difference in score for this defect was 0.5 point.

Averages of composition, yield, and fat loss data for 150 pairs of uncured cheese were as follows: clarified—moisture, 38.56 per cent; fat in dry matter, 47.7 per cent; yield, 9.65 per cent; fat in whey, 0.67 per cent; unclarified—

moisture, 39.19; fat in dry matter, 48.4; yield, 9.82; and fat in whey, 0.62. The decrease in yield resulting from clarification was apparently slightly greater than could be accounted for by the observed decrease in moisture content and the slight increase in fat loss in the whey. The lower moisture content in clarified milk cheese is undoubtedly of some importance in improving the quality, since it has been shown (24) that high moisture content is one of the factors responsible for inferior quality.

Data for 198 pairs of Swiss cheese, showing the average improvement in quality in our experiments, are presented in figure 1. A photograph illustrating the typical improvement in eye formation resulting from clarification of normal milk is presented in figure 2. The use of the clari-

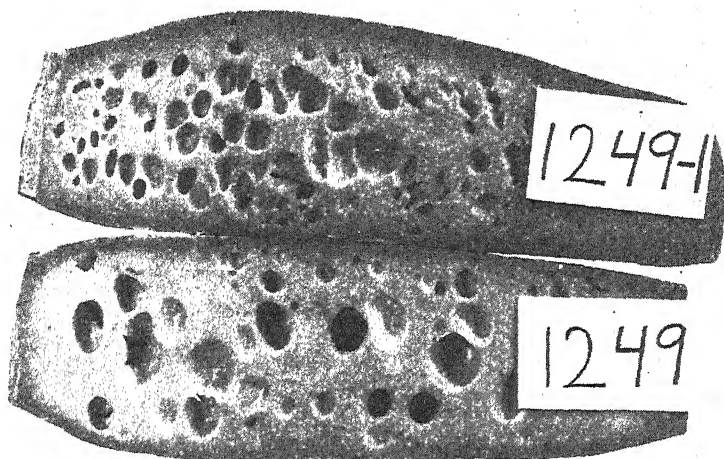


FIG. 2. Effect of clarification in improving the quality of Swiss cheese. No. 1249-1, milk not clarified. No. 1249, milk clarified.

fying process with normal milk increased the average grade from No. 2 to Special.

#### DISCUSSION

Although the removal of the extraneous matter is generally believed to be important in improving the quality of Swiss cheese, it was found in these experiments that the return of the bowl sediment to clarified milk did not injure significantly the eye formation of the cheese, although it tended to result in cheese of softer texture. The extent of leucocyte removal (about 70 per cent) by clarification was in agreement with the work of earlier investigators. The pronounced decrease in number of leucocytes, considered in conjunction with the fact that clarification resulted in an increase in the growth of the starter organisms in milk and in cheese, has suggested

the possibility that one factor responsible for relatively poor quality in unclarified-milk cheese made from milk having mastitic characteristics may be the inhibition or partial destruction of starter organisms by leucocytes. Whitehead and Cox (32) presented data which they believed indicated evidence of ability of leucocytes, particularly if present in large numbers, to retard the activity of lactic acid bacteria by phagocytic ingestion. In our work, however, microscopic examination of starter organisms grown in unsterilized, mastitis milk showed what appeared to be inconclusive evidence of phagocytosis of the lactobacilli by the leucocytes. The increase in acid development in cheese eight hours after dipping, resulting from clarification, indicates that some factor present in mastitis milk and partially removed or altered by the clarifier has a retarding effect on the growth of the lactobacilli.

The factor of agitation appears to be responsible for many of the changes resulting from clarification of the milk. Possibly, foremost among these changes is the dispersion and alteration of the fat. Results of other investigations, mentioned above, have shown that preliminary clustering of milk fat is essential to normal creaming. Our results indicate that the decrease in creaming in clarified milk is attributable primarily to a decrease in the clustering tendency. The evidence available agrees with the explanation of Hekma (8) that the change in the clustering tendency following clarification or agitation of milk results from a decrease in the amount of agglutinating material adsorbed on the surfaces of the fat globules.

Whether the improvement in the cheese following the clarification of the milk or the clarification or agitation of the gravity cream results in any degree from the reduction in the tendency of the fat to aggregate has not been determined. It is possible that when there are more large aggregates of fat, such as occur in unclarified milk, these clusters of fat may form "weak" areas in the cheese curd. Any such fat clusters containing foreign particles, which may be picked up from the milk and which are likely to contain unusually large numbers of gas-producing organisms (6), may serve as foci for abnormal eye formation in the cheese.

While the results of the experiments on the clarification and agitation of only the gravity cream indicated that the physical effect is largely on the fat, it cannot be concluded definitely that the beneficial effects are not also partially bacteriological—the breaking up and distributing of chains and clusters of organisms. Stine (29) has shown that when cream rises on milk the organisms tend to be carried upward with the fat and their numbers per milliliter in the cream layer may be 50 times as great as in the skim milk below. Schmidt (26) found an 85-fold proportion of *S. lactis* in the cream layer.

The results described above on the interchange of gases occurring during clarification and on the effects of adding oxygen and carbon dioxide to

cheese milk indicate that the effect of the process on the amounts of these gases in milk is an important factor in controlling the type of bacterial fermentation in a manner favorable to the proper ripening of the cheese.

The cheese made from clarified mastitis milk was of unusually high quality in view of the known disadvantages in the use of such abnormal milk for cheesemaking. It should be explained that the milk received regularly for this experimental work was from a herd having a rather large proportion of Jersey cows. The normal milk was therefore relatively high in solids content and the cheese made from it was relatively firm and of slightly poorer quality generally than would be expected from milk containing less solids. The effect of clarification on the experimental cheese resulted in greater improvement in the case of mastitis milk than in the case of normal milk.

The milk received in the factories, however, comes from herds which consist largely of Holsteins or of cows belonging to other breeds that produce milk of relatively low solids content. The cheese made in most factories is less firm and often tends to have a soft texture and even a weak body. The additional softening of the body resulting from mastitic characteristics of the milk is likely, on the basis of these results, to be particularly detrimental if the milk is not clarified.

#### SUMMARY

Experiments have demonstrated that clarification of milk produces a marked and consistent improvement in the quality of Swiss cheese. Studies were conducted of the properties of the cheese milk for the purpose of investigating the intermediate factors in the improvement in the cheese. Specific effects of clarification on milk include a decrease in the tendency of the fat to form aggregates upon standing, removal of a large proportion of the leucocytes from mastitis milk, an increase in the rate of multiplication of starter organisms and improvement in results of fermentation tests, an increase in concentration of oxygen and decrease of carbon dioxide, an increase in the rate at which the oxidation-reduction potential changes at 30° C., and a slight decrease in stability to alcohol.

Specific effects of the process on the properties of the cheese include a marked decrease in number and increase in size and uniformity of eyes, an increase in the firmness of the cheese and in the incidence of the glaesler defect, an increase in the rate of multiplication of starter organisms and of acid formation, a decrease in moisture content and in yield of cheese, and an increase in the fat loss in the whey.

The effects of clarification on properties of milk and on properties and quality of cheese were found to be diminished by clarifying the milk at a relatively low temperature and with a relatively slow bowl speed; they were increased by decreasing the rate of flow of milk through the bowl by

one-half, and by increasing the temperature from 21° to 32° C. The beneficial effects of clarification of milk for cheesemaking were especially pronounced in the case of mastitis milk.

Of the intermediate factors in the improvement of quality of clarified milk cheese, those that appear most significant are a decrease in aggregation of the fat globules, an increase in oxygen and decrease in carbon dioxide, improvement in effectiveness of starters, and a reduction in leucocytes when present in large numbers.

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# PREVENTION OF MILKSTONE FORMATION IN A HIGH-TEMPERATURE-SHORT-TIME HEATER BY PREHEATING MILK, SKIM MILK AND WHEY

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In the course of experiments on high-temperature short-time forewarming of milk (6) it was observed that, when the milk was reheated, the pressure required to force the liquid through the heating, holding and cooling coils was nearly constant, whereas when raw milk was heated in this equipment more and more pressure was required to maintain a constant flow. This observation seemed of such practical value as to justify an investigation of the conditions under which the effect could be obtained, and an explanation of the cause.

## EXPERIMENTAL

The equipment was, in general, the same as that described in a recent paper (6). The pump was the reciprocating type. It operated at 200 strokes per minute and had a capacity of 90 gallons per hour. The internal diameter of the stainless steel tubing through which the liquid was pumped at the uniform rate of flow of 22 feet per second was 0.18 inch. Three seconds were required to heat and 3 seconds to cool the liquid. When the holding time was 15 seconds, the total length of the tubing from the pump to the outlet at the end of the cooling coil was about 480 feet. The pressure required to pump water through this length of clean tubing at the rate of 90 gallons per hour was 2,000 pounds per square inch. The resistance to flow of more viscous liquids was, of course, greater. Under uniform conditions an operating pressure greater than that required when the tubing was clean was considered due to a coating of milk solids or the formation of "milkstone" on the inside wall of the tubing.

It is apparent that, under the above conditions, even a very thin deposit on this tubing would be reflected in the amount of force or pump pressure required to maintain a uniform flow of liquid.

When raw milk at room temperature was pumped through the system without heating there was no increase over the initial operating gage pressure.

Preheating a test liquid other than in the high-temperature equipment was done in steam-jacketed hotwells equipped with agitators. In one hotwell it was the practice to heat 40 gallons of liquid; in the other, 50 gallons. The period required to attain the desired temperature was approximately the

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same in each hotwell. To heat from 10° C. (50° F.) to 65° C. (149° F.) took 28 minutes; to 75° C. (167° F.), 33 minutes; to 85° C. (185° F.), 40 minutes; and to 95° C. (203° F.), 48 minutes. The 40 gallons of milk, skim milk or whey were cooled by pumping over a surface cooler at the rate of 2 gallons per minute. From the other hotwell the 50 gallons of milk were drawn into a vacuum pan in 6 minutes, where 10 minutes elapsed before the temperature of the vapor over the boiling milk was lowered to 40 (104° F.) to 45° C. (113° F.).

To determine the effect of a preheating treatment on the rate of development of milkstone, the preheated material was heated in the clean, high-

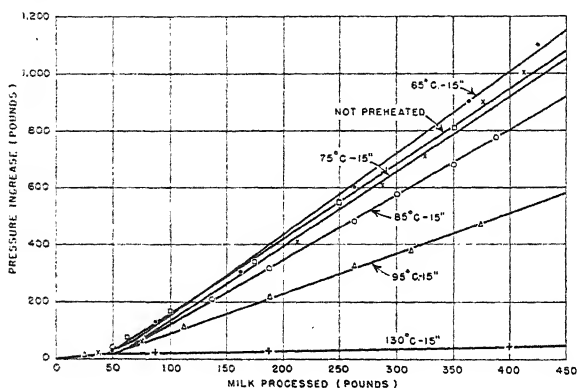


FIG. 1. Effect of preheating treatment of whole milk on the rate of pump pressure increase due to milkstone formation when the preheated milk was heated at 130° C. for 15 seconds by pumping it through heating, holding and cooling coils of 0.18 inch internal diameter. Preheating treatment is shown on each curve. Rate of milkstone formation is expressed as pounds per square inch increase in resistance to flow per pound of milk processed.

temperature equipment in 3 seconds to 130° C. (266° F.), maintained at this temperature for 15 seconds, and cooled in 3 seconds.

In making this determination, water was heated in the equipment first. When the desired temperature conditions had been established a 2-way valve was turned so that the test liquid, instead of water, would flow to the pump. One minute after this liquid began to pass into the pump the first pressure reading was noted. Additional gage readings were recorded from time to time. The last pressure gage reading was made just before the valve was turned to again admit water. The difference between the first and last readings on the pressure gage was considered the total increase in pressure.

The fresh whole milk was standardized by the Babcock test and a hydrometer reading (2) to a fat:solids-not-fat ratio of 1:2.29.

The whey was the low acid or rennet type. It was prepared from fresh skim milk by the addition of rennet and clarified.



## RESULTS

In figure 1 are shown increases in pressure gage readings due to milkstone formation on heating raw milk (not preheated) to 130° C. for 15 seconds and milk to the same temperature and for the same holding period that had been preheated in the high-temperature-short-time equipment at 65, 75, 85, 95, and 130° C. for 15 seconds. As the preheating temperature was increased less solids were deposited on the tubing. When milk that had been heated at 130° C. for 15 seconds was reheated under the same conditions only a small amount of milkstone formed.

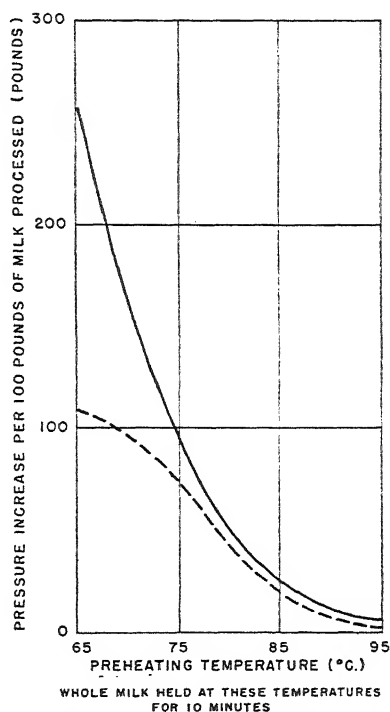


FIG. 2. Effect of preheating treatment of whole milk on the rate of pump pressure increase due to milkstone formation when the preheated milk and condensed milk made from it were heated at 130° C. for 15 seconds by pumping them through a high-temperature tubular heater. Solid line represents whole milk, broken line homogenized condensed milk of 26.0% solids content. Rate of milkstone formation is expressed as pounds per square inch increase in resistance to flow per hundred pounds of whole milk processed.

The effect of different preheating treatments of whole milk on the rate at which milk solids adhered to the tubing is shown in figure 2. In these experiments both fluid whole milk and its concentrate of 26 per cent solids content were used. The concentrated milk was homogenized at 60° C. (140° F.) and 2,500 pounds pressure before the final or test heating at 130° C. for 15 seconds.

As the preheating temperature was increased the rate at which the tubing became coated with solids decreased until, with a preheating temperature of 95° C. and a holding period of 10 minutes, the rate was slow.

In terms of whole milk equivalents, solids in the concentrated milks adhered to the tubing at a slower rate than did the solids in the preheated whole milk. However, in terms of pounds of concentrated milk processed

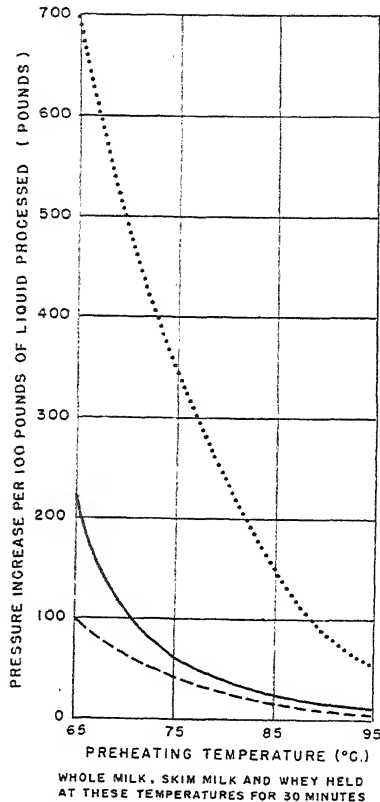


FIG. 3. Effect of preheating treatment of whole milk, skim milk and whey on the rate at which the pump pressure increased due to milkstone formation when the liquids were heated at 130° C. for 15 seconds. Solid line represents whole milk, broken line skim milk, and dotted line whey.

the rate was much faster except when the preheating temperature was below about 70° C. (158° F.) and above 90° C. (194° F.)

In obtaining the data shown graphically in figure 3 the same procedure was followed as in obtaining the data presented in figure 2 for whole milk except that skim milk and whey were used as well as whole milk and the holding period at each preheating temperature was 30 minutes.

## DISCUSSION

The formation of milkstone on dairy equipment has received much attention. It makes the transfer of heat more difficult; the deposit is insanitary, is deleterious to the metal, and must be removed. Shere (5) has described this deposit and recommends methods for removing it. He shows that its composition varies but that it is organic matter mixed with small quantities of substances which contain calcium and phosphorus.

An effective procedure for cleaning the tubing of the high-temperature-short-time equipment was to recirculate continuously through it a solution of hot trisodium phosphate and then, after flushing with water, a solution of citric acid. The pH of the former was about 11.5 and of the latter, about 2.0. In this way a large reduction in pressure was obtained in a few minutes and then, when the acid was recirculated continuously, soon there was a return to the pressure which was normal for forcing the liquid through clean tubing. If the acid was used first, only a small reduction in pressure was obtained, and usually only after a relatively long time. Finally when the acid was followed by the alkali, the tubing was cleaned quickly. This indicates that the milkstone was largely organic mixed with small quantities of inorganic matter. The proteins of milk are more soluble at pH 11.5 than at pH 2.0 and calcium phosphates are soluble at pH 2.0 but not at pH 11.5.

It is interesting to compare the relationship between the preheating treatments and the formation of milkstone with the heat denaturation of the soluble milk proteins, albumin and globulin.

Rowland (3, 4) made an investigation of the amounts of lactalbumin and lactoglobulin denatured (rendered insoluble) by heating portions of the same milk for varying periods at each of several temperatures. He found that appreciable quantities of albumin and globulin are denatured at as low a temperature as 63° C. (145° F.). In the summary of his first article he says, "Smooth curves were obtained for the progress of denaturation with time at each temperature, and, over the range of 63–75° C., the relative increase in velocity of denaturation for each rise in temperature of 1° C. was found to be constant, the temperature coefficient of the reaction being 1.5."

In the summary of his second article Rowland states, "The denaturation of albumin and globulin took place rapidly in samples of milk heated at temperatures of 75° C. and above, and was complete in approximately 60, 30, 10–15, and 5–10 minutes at 80, 90, 95, and 100° C., respectively."

Bell (1) studied the effect of heat on the solubility of the calcium and phosphorus compounds in milk. From his results he concluded that "... there is a loss in the soluble calcium and phosphorus contents of the skim milk due to heat and that the amount of the loss depends upon the temperature to which the milk has been heated.

"The results from the methods employed indicate that definitely measurable amounts of these substances are removed from solution in milks heated to 170° F. or above."

In view of the time-temperature conditions required to denature all the albumin and globulin in milk as shown by Rowland, the continued removal of calcium and phosphorus from solution due to heat as reported by Bell, and the results described in this paper, it seems probable that the formation of milkstone in a high-temperature-short-time heater can be practically prevented by suitable preheating of milk, skim milk and whey.

#### SUMMARY

1. The formation of milkstone in a high-temperature-short-time heater was greatly decreased by preheating milk, skim milk and whey.

2. There appears to be a direct relationship between the heating conditions which render insoluble some of the proteins and salts of milk and the prevention of milkstone formation.

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## LACTOSE AND ITS UTILIZATION: A REVIEW

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### INTRODUCTION

For thousands of years previous to the seventeenth century, milk was considered as having only three components, since it was common practice to remove fat and curd, either separately or together, thus leaving a third substance, whey. Whey, or serum, appears to have been used in considerable quantities by physicians of the time of Hippocrates and Galen and through the middle ages without realization that its effects were due chiefly to one specific component (8).

The first record of the isolation of the "essential salt of serum" was published in 1633 by Bartolettus (3, 11, 15), who in 1619 had written of milk as composed only of fat, serum and curd (2). Ettmüller, in 1688 (5), published improvements on Bartolettus' process of evaporation and included the purification of the crude lactose by recrystallization.

During the eighteenth century, lactose became a commercial commodity (1, 7, 10), its use being principally in medicine in place of the whey formerly used (4, 6, 8, 12, 13). The foundation of our present knowledge of lactose was laid during the nineteenth century and from this basis there has developed over the past fifty years the present structure of understanding of the characteristics and utility of this unique sugar.

By far the greatest proportion of lactose is consumed as a component of milk. The lactose isolated from milk and refined is used almost entirely in foods for infants and invalids and in pharmaceutical preparations as an

excipient. Many other uses, such as in silvering mirrors, for preserving latex and oilcake and for giving a frosty appearance to certain bottled liqueurs, are listed by writers on lactose, but these are of negligible commercial importance. Whey, because it is relatively cheap and contains readily fermentable lactose, is used as a medium for the production of lactic acid and riboflavin and has been advocated for use in the production of ethanol and penicillin.

Lactose, or milk sugar, has been found in the milks of all species of mammals, the approximate range being from 2.0 to 8.5 per cent (14). Cow's milk normally contains about 4.8 per cent. No other sugar is present in any appreciable percentage in milk, and it is probably true that the only natural process in which lactose is formed is that of lactation.

For the calculation of the lactose available annually in unprocessed whey, the data of the year 1940 may be taken as representative. In that year there were produced as a byproduct of cheese manufacture 6 billion pounds of sweet whey, of which one billion pounds was converted to whey powder. The remaining 5 billion pounds contained 225 million pounds of lactose. There were also 2 billion pounds of whey from the manufacture of casein and skim milk cheese, from which should be deducted the 200 million pounds of casein whey used in the production of 5 million pounds of refined lactose. The 80 million pounds of lactose originally present in the milk from which the remaining 1.8 billion pounds of whey was derived had been in part fermented to lactic acid, which reduced its degree of availability except for complete conversion to lactic acid. However, since sweet whey contains 5 per cent of lactose rather than the conservative 4.5 per cent used in these calculations, 300 million pounds of lactose may be considered to have been potentially available in unprocessed whey in 1940. For the war year of 1942, the corresponding quantity of lactose in unprocessed whey was over 400 million pounds.

#### STRUCTURE

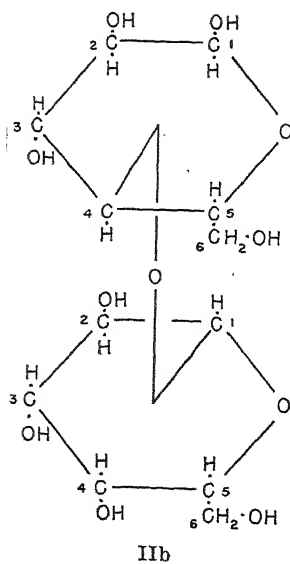
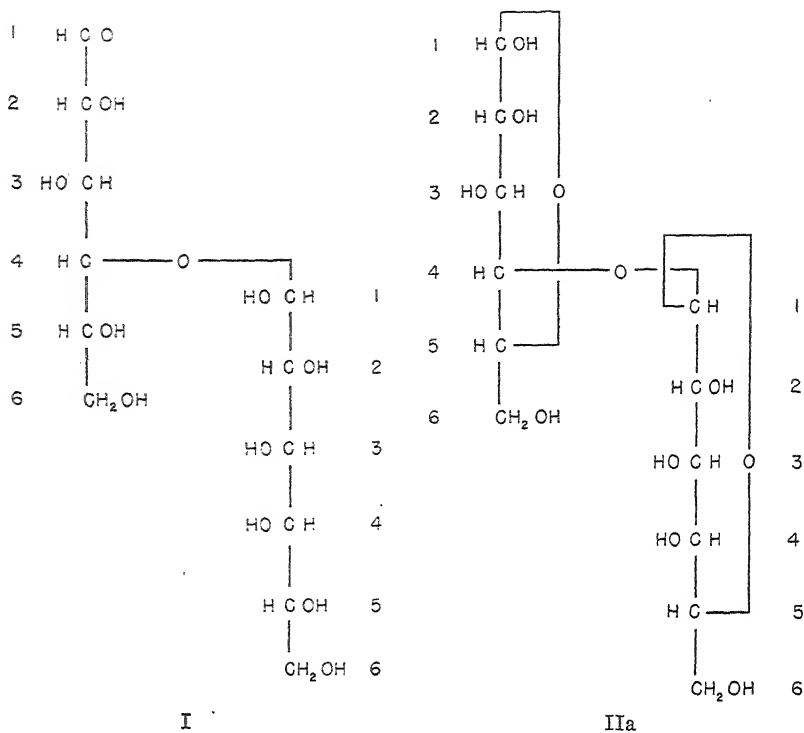
Beliefs regarding the structures of sugars have changed fairly frequently during the past fifty years and it is possible that facts yet undiscovered may change current views of the manner of linkage both within and between monosaccharides. It seems proper only to mention here chronologically the evidence that has led to the structure that is generally accepted for lactose at the present time.

In 1812, when lactose was first hydrolyzed (45), it was supposed that the substance produced was glucose only. The establishment of the proportions of the elements in lactose (44), the discovery that hydrolysis of lactose gives a sugar that is not glucose (19, 39), and the discovery of the aldehydic reducing action of lactose (37) in 1855 and 1856 led to the establishment ten years later (23) of the fact that *two* hexose sugars, galactose and glucose, are the products of hydrolysis of lactose.

In 1888, Emil Fischer hydrolyzed lactosone, obtaining glucosone and galactose (20), and in the following year he hydrolyzed lactobionic acid, obtaining gluconic acid and galactose (21). Since the reactions producing osones and monobasic acids from aldoses take place at the aldehyde end of the sugar molecule, it was concluded that the aldehydic portion of lactose is the glucose residue, and that the aldehyde group of galactose is not present as such in lactose, but must be the point of union of the galactose to the glucose residue. Hence lactose is a galactosyl glucose. This conclusion has been verified by many similar experiments.

Since one of the products of the hydrolysis of methylated lactose is 2, 3, 6-trimethylglucose (26), the point of union of the glucose residue should be on either the 4 or 5 carbon. The correct choice depends upon whether the lactone ring has a 1-4 (furanose) or a 1-5 (pyranose) linkage. Evidence as to which of these rings is normal for monosaccharides and, furthermore, as to whether the rings in polysaccharides are necessarily the same as those of the monosaccharides formed by hydrolysis has been confusing and even conflicting (17, 27, 28, 30, 32, 33, 35, 36, 46), but the pyranose structure (24) is generally accepted at present for lactose and for both the glucose and galactose components. That the glucose union is at carbon 4 has been shown by other means as well. Whether lactose, and consequently the glucose residue in lactose, has the alpha or beta configuration is easily decided, since the designation alpha is arbitrarily assigned to the form having the greater rotation in the dextro direction. That the configuration of the galactose residue in lactose is of the beta form has been shown by Fischer (22), who found that an enzyme that hydrolyzed lactose would hydrolyze a  $\beta$ -methylgalactoside, but not an  $\alpha$ -methylgalactoside, and that an enzyme that hydrolyzed an  $\alpha$ -galactoside, but not a  $\beta$ -galactoside would not hydrolyze lactose. The arrangement of the H and OH groups on the other asymmetric carbon atoms of the hexose residues is a matter on which there is general agreement and will not be discussed here, except to point out that the structural difference between glucose and galactose is in relative arrangement of the H and OH groups on carbon atom 4.

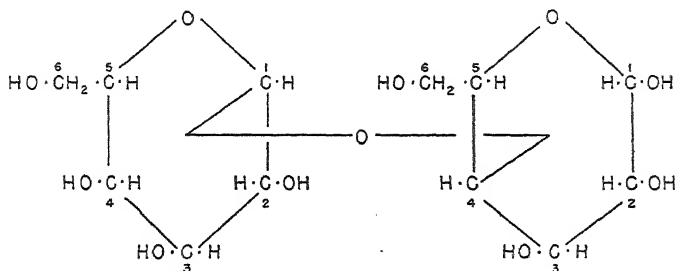
The accompanying formulas are in agreement with accepted facts. I is a formula representing in one plane the very small percentage of lactose believed to be present in lactose solutions in the form of a chain, with a functional aldehyde group at carbon 1 of the glucose residue. IIa shows in one plane the 1-5, or pyranose rings, of the predominant, lactonic form (27). IIb is a formula of the Haworth type (30), indicating the relative positions of component groups in three-dimensional space. Atoms placed above the carbon atoms are to be considered as on one side of the plane of the ring, those below on the other, the rings themselves being in different planes. A recent modification (18) of the Haworth scheme of representation is shown as IIc. In this scheme the O atoms of the lactone rings are placed at the top.





atoms at the right of carbon atoms are considered to be below the plane of the ring, those at the left above. In comparing these formulas, it should be noted that the numbering system is the same in all four, and that IIa, IIb, and IIc differ only in the method of representation. All four formulas are supposed to represent  $\alpha$ -lactose; interchange of the positions of the H and OH on carbon atom 1 of the glucose residue or the corresponding change in the position of the lactone ring of the glucose residue converts the formula to that of  $\beta$ -lactose.

An anhydrous modification (31) containing five moles of  $\alpha$ -lactose to three moles of  $\beta$ -lactose has been prepared by crystallization from methanol. Epilactose has been prepared from lactose (25) and differs from it in the reversal of the positions of H and OH on carbon atom 2 of the glucose residue, being therefore a galactosyl mannose. Neolactose (34) differs from lactose in that the H and OH groups on both carbon atoms 2 and 3 of the glucose residue are reversed in position. It is, therefore, a galactosyl altrose. Lactulose (38) is a galactosyl fructose.



IIc

Gynolactose, a levorotatory galactosylglucose, and allolactose, a dextro-rotatory galactosylglucose, have been isolated in small quantities from human milk (41, 43). Allolactose is believed to be 6- $\beta$ -d-galactosyl-d-glucose (29, 42), presumably differing in structure from lactose only in that the galactose residue is linked to carbon 6 of the glucose residue rather than to carbon 4.

#### PHYSICAL FORMS AND EQUILIBRIA

Mutarotation is a phenomenon characteristic of all natural reducing sugars in water solution and in such instances is attributable to changes in the proportion of alpha and beta forms. The mutarotation of lactose solutions was first noted by Erdmann in 1855 (48). Several relevant observations were reported previous to 1900 (47, 49, 59, 60, 61, 63), but the most extensive investigations of mutarotation of lactose solutions and of equilibria among the forms of lactose, both in solution and in the solid state, have been carried out more recently by Hudson (52, 53, 54), by Gillis (50, 62), and by Parisi (57).

The ordinary lactose of commerce has the composition expressed empirically by the formula  $C_{12}H_{22}O_{11} \cdot H_2O$ . It has the specific optical rotation in water solution  $[\alpha]_{20}^D = +89.4^\circ$ , a melting point of  $201.6^\circ C.$ , and is a monohydrate of 4- $\beta$ -d-galactosyl- $\alpha$ -d-glucose, being designated more briefly as  $\alpha$ -lactose hydrate.

When  $\alpha$ -lactose hydrate is dissolved in water, its specific rotation changes gradually from  $+89.4^\circ$  to  $+55.5^\circ$  at ordinary temperatures, the rate of change being a function of the temperature, of the concentration of H and OH ions in the solution and of the concentration of certain other solutes.

If an aqueous lactose solution, however prepared, is concentrated and crystallization is caused to take place at a temperature below  $93.5^\circ C.$  and at a moderate rate, the crystals formed are  $\alpha$ -lactose hydrate. If, however, the temperature during crystallization is above  $93.5^\circ C.$ , the crystals have the composition  $C_{12}H_{22}O_{11}$ , a specific rotation in water solution  $[\alpha]_{35}^D = -35.0$  and a melting point of  $252.2^\circ C.$  This form is 4- $\beta$ -d-galactosyl- $\beta$ -d-glucose and is usually designated  $\beta$ -lactose anhydride or, more simply,  $\beta$ -lactose.

When  $\beta$ -lactose is dissolved in water, its specific rotation changes gradually from  $+35.0^\circ$  to  $+55.5^\circ$ , the solution ultimately becoming identical in all respects to one prepared from  $\alpha$ -lactose hydrate and water in corresponding proportions. The equilibrium rotation of  $+55.5^\circ$  is attained practically instantaneously in lactose solutions at temperatures above  $70^\circ C.$ , whether the lactose dissolved was alpha or beta.

If  $\alpha$ -lactose hydrate is dehydrated by heating at a temperature below  $93.5^\circ C.$ , preferably under reduced pressure and not below  $65^\circ C.$ , a variety having the composition  $C_{12}H_{22}O_{11}$  is formed. Its melting point is  $222.8^\circ C.$  When it is dissolved in water, the solution manifests the same rotation and mutarotation as one containing the corresponding quantity of the hydrate, and therefore this compound is designated  $\alpha$ -lactose anhydride. This anhydride is stable in dry air, but in the presence of moisture it changes to  $\beta$ -lactose anhydride at temperatures above  $93.5^\circ C.$ , to  $\alpha$ -lactose hydrate at temperatures below  $93.5^\circ C.$

A solution of lactose in a state of rotational constancy, or equilibrium, at  $25^\circ C.$  has 62.25 per cent of its lactose in the  $\beta$  form and 37.75 per cent in the  $\alpha$  form. The equilibrium constant is therefore 1.65 at this temperature. This ratio is unaffected by changes in pH value of the solution, but is altered by changes in temperature, being 1.62 at  $20^\circ C.$  and 1.81 at  $49^\circ C.$  according to Kendrew *et al.* (55). According to Gillis (50) K decreases with rise in temperature, being 1.65 at  $0^\circ C.$  and 1.33 at  $100^\circ C.$

If an equilibrated solution of lactose is practically instantaneously deprived of its water, as may be done by a spray or drum dryer, the resulting solid is an amorphous glass containing the  $\beta$  and  $\alpha$  forms in the equilibrium

ratio. Drying more slowly above 93.5° C. increases the ratio of  $\beta$  to  $\alpha$  and can even result in practically pure  $\beta$ -lactose.

The velocity constant of approach to equilibrium in solution,  $k_1$  (for  $\alpha$  to  $\beta$ ) +  $k_2$  (for  $\beta$  to  $\alpha$ ), is strongly affected by changes in temperature and in the concentrations of components of the solution, particularly H and OH ions. Hudson found the relative percentages of  $\alpha$  transformed in one hour to be 3.4 at 0° C., 17.5 at 15° C., and 51.1 at 25° C. At 70° the change is practically instantaneous. These rates should be remembered when evaluating claims of differences in properties of  $\alpha$ - and  $\beta$ -lactose determined by comparisons made on solutions of the two forms, particularly when such solutions have stood for some time at an elevated temperature.

An equation has been derived by Parisi (57) for the velocity constant of the  $\beta$  to  $\alpha$  change,  $k_2 = ax^{pH} + by^{pOH}$ , in which  $a$  and  $b$  are coefficients depending on temperature and  $x$  and  $y$  are values of the rate of change as a function of OH-ion and H-ion concentrations, respectively.  $k_2$  is at a minimum at approximately pH 5.0 and the effects of the concentrations of H and OH ions are equal at approximately pH 7.0. Since the effect of OH-ion concentration is much greater than that of H-ion concentration,  $k_2$  increases more rapidly with increase of pH above 5.0 than with decrease of pH below 5.0. Since the ratio between  $k_2$  and  $k_1$  is very nearly an absolute constant, an analogous equation may be written for  $k_1$ . In applying these equations, it is necessary, in converting values of pOH to equivalent values of pH, to take into account the variation of the value of  $K_w$  with temperature (51).

Values of  $k_1$  and  $k_2$  may be calculated from experimentally determined values of  $k_1 + k_2$ , since  $k_1/k_2$  is determinable from the ratio of the components of an equilibrated solution.

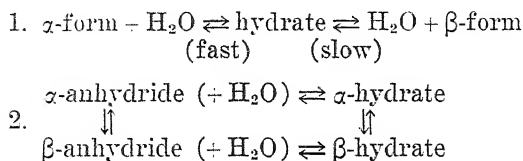
An empirical equation has been formulated by Herrington (51) for calculating the mutarotational constant in terms of pH values:  $\log (k_1 + k_2) = 0.00415 (\text{pH} - 4.45)^4 - 0.34$ . This equation is applicable only at 25° C. and in the absence of catalytic substances other than H and OH ions.

The presence of lactate or of acetate in normal concentrations at pH values of 4.0 to 5.0 increases the value of  $k_1 + k_2$  from 4 to 8 times. Presumably other weak acids have similar catalytic effects.

Several conclusions may be drawn from the above facts. It is not proper to consider the hydrate of lactose as neither  $\alpha$  nor  $\beta$ ; but, since the hydrate and  $\alpha$ -lactose anhydride have the same molecular rotation when freshly dissolved and show the same mutarotational changes, they are properly classed together as of the  $\alpha$  variety, in contrast to the  $\beta$ -anhydride, which has a different molecular rotation. A saturated aqueous solution of lactose at 93.5° C. is saturated with respect to both the  $\alpha$  and  $\beta$  forms. This does not mean that the two forms are present in equal quantities; the equilibrium persists. A saturated solution at temperatures above 93.5° C. is saturated with respect to  $\beta$ , but not with respect to  $\alpha$ . A solution saturated at temperatures below

93.5° C. and in  $\alpha$ - $\beta$  equilibrium is saturated with respect to  $\alpha$ , but not to  $\beta$ . Though  $\beta$ -lactose hydrate has not been prepared, it can be predicted that it would be more highly soluble than the  $\beta$ -anhydride and, in the solid form, would be unstable, changing to the  $\beta$ -anhydride above 93.5° and to the  $\alpha$ -hydrate below 93.5° C. The transition point for solid  $\alpha$ -lactose hydrate  $\rightleftharpoons$  solid  $\beta$ -lactose anhydride is 93.5° C. A transition point for solid  $\alpha$ -lactose anhydride  $\rightleftharpoons$  solid  $\beta$ -lactose anhydride cannot exist below the melting points of the two forms.

Two representations have been offered for the equilibria in lactose solutions (50, 54) :



Objections have been raised to the first on the grounds that it postulates relative speeds of equilibration that must be considered reversed in magnitude for the analogous equilibria for maltose, and that, since the hydrate is assumed to have a terminal group  $\text{—CH(OH)}_2$  and therefore to be neither  $\alpha$  nor  $\beta$ , it contradicts the existence of the lactonic structure for the hydrate and the asymmetry of the terminal carbon atom. Both representations are open to objection in that the assumption is made that lactose exists in solution in both the anhydrous and monohydrated forms, three forms in the first, four in the second, and, by implication, in no other degree of hydration. Freezing-point measurements have indicated that lactose in solution may be hydrated with an average of 3 to 6 molecules of water per molecule of lactose, the precise number depending on the concentration of the solution (66). In any case, the assumption that the degree of hydration of solid forms is an index to the degrees of hydration of forms in solution lacks adequate support. On the basis of current knowledge, inclusion in the representation of equilibrium in solutions of lactose of details involving hydration seems unjustifiable.

#### SOLUBILITY AND CRYSTALLIZATION

The solubility of lactose in water is shown in table 1, which contains experimental and calculated values of Hudson (72), of Gillis (68) and of Leighton and Peter (74). The value for the initial solubility of  $\beta$ -lactose at 0° C. is the result of a direct determination, that at 100° was calculated from the final solubility of lactose at 100° on the assumption that the equilibrium constant  $K$  equals 1.50 and that the solubility of one form of lactose is unaffected by the presence of the other form in the solution. The values for initial solubility of  $\alpha$ -lactose above 25° C. were calculated on the basis of the same assumptions. Gillis claims to have shown that the solubility of either

form is unaffected by the presence of the other and, by theoretical calculation and by experiment, to have proved that the equilibrium constant  $K$  decreases with rise in temperature. Kendrew and Moelwyn-Hughes (55), on the other hand, have both calculated and determined values of  $K$  that increase with rise in temperature. Until one can be sure as to the effect of temperature on the value of the equilibrium constant, the magnitude of mutual solubility effects will be uncertain.

The values of final solubility are the same regardless of the form initially dissolved and refer to solutions in  $\alpha$ - $\beta$  equilibrium and saturated with the  $\alpha$  form if below 93.5° C., with the  $\beta$  form if above 93.5° C.

TABLE 1  
*Solubilities of lactose*

Temperature	Initial solubility		Final solubility	Supersolubility
	$\alpha$	$\beta$		
°C.	<i>g. per 100 g. water</i>	<i>g. per 100 g. water</i>	<i>g. per 100 g. water</i>	<i>g. per 100 g. water</i>
0	5.0	45.1	11.9	25
15.0	7.1	.....	16.9	38
25.0	8.6	.....	21.6	50
39.0	12.6	.....	31.5	74
49.0	17.8	.....	42.4	.....
59.1	.....	.....	59.1	.....
63.9	.....	.....	64.2	.....
64.0	26.2	.....	65.8	.....
73.5	.....	.....	84.5	.....
74.0	34.4	.....	86.2	.....
79.1	.....	.....	98.4	.....
87.2	.....	.....	122.5	.....
88.2	.....	.....	127.3	.....
89.0	55.7	.....	139.2	.....
100.0	.....	94.7	157.6	.....
107.0	.....	.....	177.0	.....
121.5	.....	.....	227.0	.....
133.6	.....	.....	273.0	.....
138.8	.....	.....	306.0	.....

The supersolubility values for lactose are definite and readily reproducible. In general the supersolubility at any temperature is equal to the saturation value at a temperature 30° C. higher. The practical significance of supersolubility lies in the fact that solutions supersaturated to a degree not exceeding the supersolubility value will not crystallize, even if the solution is agitated, unless lactose crystals or particles of some isomorphous substance are introduced. Even then, a general crystallization may not occur, but only growth of the nuclei ensue. Herrington (70) has demonstrated that solutions saturated with lactose at approximately 50° C. may be cooled to 0° C. without spontaneous crystallization taking place, provided the solutions are not agitated.

It should be borne in mind that solutions of lactose supersaturated to more than a slight degree may be supersaturated to both  $\alpha$ - and  $\beta$ -lactose. From such solutions, either form may be made to crystallize initially by seeding with the desired variety. If the form initially separating is the one more soluble at the temperature of the solution, a subsequent seeding with the less soluble form will cause the less soluble form to crystallize and the more soluble form to redissolve until both solubility and  $\alpha$ - $\beta$  equilibria are established in the solution.

In the crystallization of lactose at ordinary temperatures from a solution seeded with the  $\alpha$ -hydrate, two factors are involved—the degree of supersaturation and the rate of change of  $\beta$  to  $\alpha$  tending to maintain equilibrium. The rate of crystallization has been studied at temperatures in the range of  $-5^{\circ}$  C. to  $-30^{\circ}$  C. (77) and it has been found that for the first two and one-half hours, the rate of crystallization is greater at  $30^{\circ}$  C. than at  $25^{\circ}$  C. or at any lower temperature. The quantity of lactose separated is greater at  $30^{\circ}$  C. than at any lower temperature until about seven hours have elapsed. The most efficient method of crystallizing lactose should be to cool the solution to  $30^{\circ}$  C., to seed it and stir it at  $30^{\circ}$  for about 3 hours, then to cool it to near  $20^{\circ}$  C. and to hold it at that temperature for 3 or 4 hours or until a convenient time to filter. There is a definite disadvantage in crystallizing at temperatures below  $20^{\circ}$  C.

In a nearly saturated sucrose solution the solubility of lactose is about one-half what it is in pure water (75). Lactose in solution is about one-sixth as sweet as sucrose (67), but the solid sugar seems even less sweet because of its comparatively low solubility and the hardness of its crystals. Solid lactose produces on the tongue a sensation similar to that produced by sand; in fact, the so-called "sandiness" of ice cream is caused by the presence of lactose crystals.

Lactose is insoluble in 95 per cent ethyl alcohol, in methyl alcohol, and in ethyl ether. Pyridine saturated with lactose at  $25^{\circ}$  C. contains 2.18 per cent of the sugar (71). Lactose may be dissolved in warm acetic acid, either dilute or glacial, from which it separates unchanged on cooling (76).

#### OTHER PHYSICO-CHEMICAL DATA

Lactose acts as a weak polybasic acid, binding two moles NaOH per mole of sugar and possibly more. Values for the apparent acid dissociation constants according to several investigators are: the first constant,  $1.05 \times 10^{-12}$ ;  $0.76 \times 10^{-12}$ ;  $1.20 \times 10^{-12}$ ; the second,  $3.6 \times 10^{-14}$ ,  $3.0 \times 10^{-14}$ ,  $3.63 \times 10^{-14}$ ; the third,  $1.7 \times 10^{-14}$ ; the fourth,  $1.6 \times 10^{-14}$  (84, 93, 96).

The coefficient of cubical expansion of lactose is 0.00911 per degree between  $0^{\circ}$  C. and  $100^{\circ}$  C. (87). The specific gravity of the  $\alpha$ -hydrate is 1.5453 (81), that of the  $\beta$ -anhydride 1.59. The specific gravity of lactose solutions is not a straight-line function of concentration, being affected by a contrac-

tion which has a maximum value of 0.593 ml. per 100 grams of solution at 20° C. at a concentration of 54.03 per cent. Such a solution contains 18 moles  $\text{H}_2\text{O}$  per mole  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ .

The refractive indices of the tomahawk-shaped crystals of  $\alpha$ -hydrate are given as  $\alpha = 1.517$ ,  $\beta = 1.542$  and  $\gamma = 1.550$  (89), and as  $\alpha = 1.517$ ,  $\beta = 1.553$  and  $\gamma = 1.555$  (97). The refractive indices of crystals of  $\beta$ -lactose are given as  $\alpha = 1.542$ ,  $\beta = 1.572$  and  $\gamma = 1.585$  (97). The ratios of the axes of crystals of  $\alpha$ -hydrate are given as  $a:b:c = 1:0.6215:0.2193$  (92) and as  $a:b:c = 1:0.3677:0.2143$  (95). The ratios for the axes of crystals of  $\beta$ -lactose are given as  $a:b:c = 0.817:1:0.377$  (97). The values mentioned are for crystals of the forms usually seen. However, the crystal habit of each variety of lactose is influenced greatly by conditions under which crystallization takes place. The different types of lactose crystals are discussed in detail by Hunziker and Nissen (86) and by Herrington (83).

The specific heat of the hydrate is 0.299, that of the  $\beta$  form 0.2895; the molecular heat of the solid hydrate is 107.6 cal., that of the solid  $\beta$  form 99.0 cal.; the apparent heat of either form when it is dissolved in water is 147 cal. (90). Other thermochemical data are given in table 2. Extensive

TABLE 2  
*Thermochemical data on lactose. 20° C.*

Heat of	$\alpha$ -hydrate		$\beta$ -anhydride		$\alpha$ -anhy- dride	Ref.
	<i>cal. per gram</i>	<i>Cal. per mole</i>	<i>cal. per gram</i>	<i>Cal. per mole</i>	<i>cal. per gram</i>	
Combustion .....	3945.0	1420.0	4162.0	1423.0	.....	91
“ .....	3736.8	1345.2	3951.5	1351.4	.....	94
“ .....	3761.6	1354.7	3932.7	1345.5	.....	79
“ .....	3948.0	1421.0	.....	.....	.....	88
Formation from elements .....	.....	610.8	.....	535.6	.....	94
“ “ “ .....	.....	593.5	.....	534.4	.....	79
Solution, initial .....	-12.0	.....	-2.3	.....	+7.3	85
“ final .....	-11.4	.....	-2.7	.....	+7.9	85
Transition to $\beta$ .....	+1.0	.....	.....	.....	+1.0	85
Transition to equilibrium mixture .....	.....	.....	.....	.....	+1.3	85

data on heat capacity and entropy of lactose will be found in papers by Anderson and Stegeman (78) and Furttsch and Stegeman (82).

#### MANUFACTURE OF LACTOSE

*General considerations.* The refined lactose of commerce is a white, odorless powder consisting of crystals that have been pulverized sufficiently to pass a No. 100 screen. It is at least 99.7 per cent pure as determined by the polariscope and contains not more than 0.050 per cent ash, not more than 0.020 per cent nitrogen and not more than 0.020 per cent fat. It complies with the U.S.P. heavy metals test and its solution in water is clear, colorless,

odorless and neutral to litmus paper. More stringent standards are required for bacteriological and other special uses.

For use in modifying milk or as a substrate for fermentation processes, lactose containing comparatively large percentages of the other components of whey is not ordinarily objectionable, and in some instances the presence of these substances is desirable. However, lactose containing excessive quantities of nitrogen compounds develops unpleasant odors if stored for any considerable time under ordinary conditions of humidity and temperature, and this fact should be borne in mind when the cruder grades of lactose are being produced for other than immediate use.

The whey remaining as a byproduct from the manufacture of casein by precipitation with hydrochloric acid is the source of nearly all the lactose produced in the United States. The whey from casein precipitated by means of sulfuric acid is objectionable because of the difficulty of removing slightly soluble sulfates that impart cloudiness to lactose solutions. The whey from casein precipitated by the lactic acid produced by bacterial fermentation of part of the lactose in the milk is not used because of the relatively low percentage of lactose left in the whey. Lactose can be made successfully from sweet whey derived from the manufacture of cheese or rennet casein, the processes differing slightly from that employed when casein whey is used.

Whey contains on the average 5 per cent lactose, a small fraction of a per cent of fat and 1.6 per cent of protein plus ash. In casein whey there is usually about 0.85 per cent protein and 0.75 per cent ash, in rennet-cheese whey 1.10 per cent protein and 0.50 per cent ash. The difference in the percentages of ash is attributable mainly to the calcium phosphate which is present in casein whey in appreciable quantities, but in rennet whey in practically negligible quantities. Rennet whey contains a small percentage of acid-precipitable protein.

If casein whey is concentrated there is obtained a viscous, sirupy mass from which lactose will not crystallize (104). If the whey is adjusted to neutrality from its initial pH value of between 4.0 and 5.0 and then concentrated without filtration, crystallization is still difficult. In order to obtain efficient crystallization of lactose from casein whey, it is necessary to remove the neutralization precipitate, which consists largely of the calcium phosphate mentioned above, and it is desirable to remove the heat-coagulable protein. Rennet whey when adjusted to neutrality forms no neutralization precipitate.

The facts given above are necessary to an understanding of some of the procedures employed in obtaining crude lactose from whey.

*Manufacture of crude lactose.* Casein whey is heated in iron tanks with live steam to the boiling temperature (117, 121). During the heating, milk of lime is added gradually until the pH value of the whey is 6.2, or the titrat-



able acidity is about 0.05 per cent, expressed as lactic acid. Excess of lime should be avoided. It is not necessary to boil the whey, since the coagulation of the heat-precipitable protein will be complete at slightly below the boiling point. The batch is allowed to stand for a few minutes to allow the coagulum of protein and calcium phosphate to separate from the clear liquid.

The clear whey is drawn to a storage vat that feeds to a double-effect vertical evaporator, the sludge being held back for filtration later. Evaporation of the water is continued until the whey has a concentration of 20° Baumé, or 30 per cent lactose. Foaming during evaporation may be minimized by having the evaporating pan clean at the start and by adding foam-reducing oils.

The thin sirup from the evaporator is filtered through cloth in a filter press and is followed by the sludge from the coagulation vat, the press thus being cleaned of adhering sirup. The wash whey may be combined with the filtered sirup, but preferably is run to the storage vat for clarified whey.

The thin sirup is next concentrated further in a single-effect vacuum pan, in which it is brought finally to a concentration of about 40° Bé., the exact concentration being determined by experienced operators by observing the degree of crystallization, or "graining," that has taken place. Toward the end of the graining process, some operators add hydrochloric acid, principally to diminish foaming, but also to prevent darkening of the sugar and precipitation of salts.

The hot mass is dropped from the pan into crystallizing vats, in which crystallization may be allowed to continue for several days. It is usually considered preferable to employ crystallizers equipped with slow-speed agitators and jackets in which cold water can be circulated. In these, the temperature of the crystallizing mass can be brought in a short time to about 30° C. and later still lower. By controlling the amount of agitation and the rate of cooling, it is possible to obtain uniform crystals of any desired size.

When crystallization is complete, the soft, wet mass is run to a sugar centrifuge, where the crystals are freed from mother liquor and washed with cold water. The washings are run to the vat for clarified whey and the mother liquor may be concentrated to obtain a second crop of crystals. Because of increased viscosity of this second sirup due to accumulation of salts and other substances, it is somewhat difficult to separate the second crop of crystals. The second mother liquor is discarded.

The wet, crude lactose should either be refined promptly or dried in a tunnel dryer to avoid spoilage.

The first crop should comprise about 70 per cent of the sugar present in the whey and contain between 85 and 90 per cent lactose. It will be slightly yellowish in color.

If so desired, cheese whey may be used by the procedure described for casein whey, provided the whey is evaporated to a thin sirup of about 18° Bé. concentration before, rather than after, liming and heating.

If a sweet whey, such as that from the making of Swiss cheese, is used, the costly operations of boiling and preliminary evaporation of the whey and the filtration of the thin sirup and sludge may be avoided (102, 103, 116). The whey is neutralized to a pH value of about 7.0, or a titratable acidity, expressed as lactic acid, of 0.04 per cent, evaporated under vacuum to a concentration of 32° Bé. (read at 50° C.), or 62 per cent total solids, and the lactose grained and crystallized. The filtrate from the centrifugal separation of the sugar may be dried, preferably in a spray dryer, to produce a whey powder relatively low in proportion of lactose and high in soluble protein, milk salts and riboflavin. This product is highly desirable for modifying milk and for general food use.

The procedure for obtaining crude lactose may be modified in such a way that a sugar suitable for some technical uses may be made by only one crystallization (127). Such a lactose contains very little protein and forms in water a clear but slightly yellowish solution that does not foam appreciably on boiling. After the hot, limed whey has been separated from the coagulum of protein and calcium phosphate, its temperature is regulated to the range of 56° to 58° C. and one part of trypsin is added for each 10,000 parts of whey. The action of the enzyme is allowed to proceed for about an hour, the temperature being held between 56 and 58° C. At the end of the holding period, the protein will be broken down into more soluble units which will not contaminate the sugar when it is crystallized later. A sample of the treated whey should not show precipitation when trichloro-acetic acid is added. The whey is evaporated to 20° Bé. and the sirup decolorized, about one pound of carbon being required for each 1000 pounds of sugar in the sirup. It is then filtered, evaporated to 40° Bé., and the sugar grained, crystallized, centrifuged, washed and dried as previously described.

Lactose has also been made as a byproduct of a process conducted primarily for the purpose of reducing the lactose content of skim milk that is to be used in the manufacture of ice cream (113, 126). This procedure is based on research of Leighton and Leviton (112), which demonstrated that the addition of cane sugar to skim milk before condensing lessens the viscosity of the concentrated milk to such a degree that crystallization of lactose takes place readily and the separation of the crystals by mechanical means is easily accomplished.

The procedure recommended is the following: 5.9 pounds of cane sugar is added to each 100 pounds of skim milk, the mixture is forewarmed to 63° C. for 10 minutes, concentrated under vacuum to 70 per cent total solids, transferred to a crystallizing vat, cooled to 25° C., held over night and the crystallized lactose separated by means of a centrifuge or filter press. About 65 per cent of the lactose in the skim milk is removed by this procedure. The crystals are coated with protein and require a refining treatment.

A process of extracting lactose from spray-dried whey has been devised by Leviton (114, 115). The dried whey should be freshly made or should have been stored in a cool, dry place, since the success of this procedure depends on the lactose being in the amorphous state in the powder. The dried whey is stirred rapidly into 15 times its weight of 76 per cent (by volume) ethanol and, after three minutes, the insoluble protein is removed by filtering through a filter press. The filtrate is acidified to a reaction of about pH 3.6 and allowed to crystallize over night. The lactose is removed by means of a centrifuge. Eighty per cent of the lactose in the first filtrate, or 75 per cent of that in the dried whey, is obtained as a white sugar of a high degree of purity. If 95 per cent ethanol is used, the yield is somewhat less and the sugar is in the form of the equilibrium mixture.

Several other methods of separation of lactose have been advocated, such as the drying of whey, followed by fractional extraction with water (109, 111); the freezing out of water from whey below 0° C., followed by crystallization of the lactose at 0° C. (106) or extraction with alcohol (108); and the drying of clarified whey (125). Many patents have been issued on special methods of clarification of whey.

*Refining of lactose.* For refining (117, 122), the crude sugar is dissolved with the aid of live steam in sufficient water to give a concentration of 20° Bé. To this sirup are added one-fourth pound of a filter aid and one pound of decolorizing paste per 100 pounds of crude sugar in the batch. The decolorizing paste consists of four parts decolorizing carbon, one part hydrochloric acid and enough water to make the paste easy to handle. The batch is heated to boiling and sufficient hydrochloric acid added to produce a titratable acidity of 0.09 per cent, expressed as lactic acid. It is desirable at this point to allow the batch to stand over night in order that the decolorization process shall reach its maximum effectiveness. The following morning the solution is reheated to near the boiling point and sufficient milk of lime cautiously added to reduce the acidity to 0.05 per cent, or to give a pH value of between 5.4 and 5.8. An experienced operator can judge the proper reaction by the degree of flocculation. The solution is then boiled vigorously for a few minutes and allowed to stand until the flocculated mixture of carbon, protein and insoluble salts has settled. It is then filtered, first through cloth in a filter press and again through a sheet of fine-mesh, rag paper supported between two perforated copper disks. If the filtered sirup is cloudy, insufficient lime was used; if the filtration is slow or the solution is colored, too much lime was used.

The filtered solution is acidified with hydrochloric acid to ensure that the salts and protein still present will remain in solution, then evaporated to 40° Bé., and the sugar grained, crystallized, centrifuged and washed with cold water as in the process for crude sugar. The mother liquor is run to the vats for storage of 20° Bé. sirup for crude sugar production. The wash waters are used for dissolving crude sugar for refining.

The wet lactose, after being tested to make sure that it is of the desired purity, is dried in a tunnel or a rotating drum at a temperature of approximately 80° C. From two to three hours are required. The air circulated to the dryer should be filtered through oil-treated glass wool to remove any dust particles that may be present.

The dried sugar is transferred to covered hoppers from which it goes to a pulverizer, where it is ground until practically all will pass a No. 100 screen. The lactose is packed in barrels having two liners, the one next to the wood being of waterproof paper, the one next to the sugar being of fine, unbleached muslin.

The yield of refined lactose is ordinarily expected to be not less than 50 per cent of the sugar present in the whey, but skillful operation of the refining steps will increase the overall yield to 60 per cent.

*Manufacture of beta lactose.* Because beta lactose is initially more soluble than alpha lactose, there has developed a demand for beta lactose that has been met by the devising of several practical processes for the conversion of alpha lactose partially or entirely to the beta form.

Drying lactose solutions by the spray process produces a mixture of the alpha and beta forms in approximately the equilibrium ratio (105). This mixture is more soluble than alpha lactose initially, but less soluble than the mixture of the two forms in the ratio of their individual solubilities. It has a high rate of solution because of its fine amorphous condition, but is hygroscopic and has poor wetting properties.

A sugar containing between 90 and 99 per cent of the beta form may be made by drying lactose solutions on a drum dryer. The product is somewhat less amorphous in appearance than spray-dried lactose, is much less hygroscopic and has relatively good wetting properties. Bell (105) recommends drying a preheated 80 per cent solution of lactose on an atmospheric drum dryer with a steam pressure of 65 to 75 pounds and a drum speed of 5 to 7 r.p.m. Under these conditions lactose containing over 90 per cent of beta sugar has been produced. The critical factors of speed and temperature would probably have to be determined experimentally for any specific drying unit. Increasing the speed of drying beyond a certain rate decreases the proportion of beta lactose in the dried product.

The process of Supplee and Flanigan (123) consists of drying a lactose solution in the form of a film on a heated surface at a temperature above 100° C. and removing the film from the surface while it is still a paste containing at least 2 per cent water. The lactose crystallizes in the beta form and the heat remaining in the paste completes the drying.

Sharp's earlier process (118) consisted of adding alpha lactose to a saturated solution maintained at a temperature above 93.5° C. and removing an equivalent quantity of lactose in the beta form. Since a solution super-

saturated with lactose above  $93.5^{\circ}\text{C}$ . is more highly supersaturated with respect to the beta form than to the alpha and since equilibrium between the two forms in solution is established very rapidly at that temperature, the beta form crystallizes readily and, at saturation (undersaturation with respect to alpha), alpha can dissolve and will reappear as crystals of beta. By filtration in a heated centrifuge, a sugar containing a high percentage of beta lactose is obtained.

More recently, Sharp and Hand (119) have developed a procedure whereby dry alpha lactose hydrate is heated in a closed container to  $120^{\circ}$ – $130^{\circ}\text{C}$ . In the presence of the water vapor formed, a solution of alpha lactose forms on the surface of the crystals and beta lactose crystallizes from this solution. When the conversion is complete, or at a desired stage short of completeness, the water vapor is allowed to escape.

The method of Verschuur (110, 124) has apparently not been tried on a large scale, but is of interest because of the claim made that the beta lactose contains no alpha and because of the unusual procedure employed. Pyridine is added to a boiling solution of lactose in water. Pyridine and water are distilled off and then more pyridine is added and the distillation continued. Beta lactose crystallizes from the solution as the proportion of water present becomes small. The crystals are washed with boiling pyridine and finally with hot ethanol and dried.

#### HYDROLYTIC PRODUCTS

Lactose may be hydrolyzed by lactase from the wall of the small intestine of mammals (131, 133, 134, 135, 137, 139), by emulsin from almonds (134), by lactase secreted by lactose-fermenting yeasts (128, 129, 130, 132, 142, 143) and by dilute solutions of strong acids (136, 138, 140, 141). Glucose and galactose in equal quantities are the immediate products of the hydrolysis. The enzymatic hydrolysis of lactose has not been developed commercially, largely because of the difficulty of obtaining an adequate supply of enzyme.

The rates of hydrolysis of the different forms of lactose have been found to be the same (141). The half-period of hydrolysis of lactose in 3 per cent solution in 0.05 molar hydrochloric acid at  $98^{\circ}\text{C}$ . is 125 minutes; in 18 per cent solution, 94 minutes (136). A 10 per cent solution containing only sufficient hydrochloric acid to give a pH value of 1.2 to 1.3 is practically completely hydrolyzed at  $150^{\circ}\text{C}$ . in an hour. (138). A sirup made under these conditions, concentrated and neutralized has a pleasing flavor and no objectionable saltiness. If concentrations of lactose greater than 10 per cent are hydrolyzed by this procedure, other products, some of which have a bitter taste, are produced at the expense of part of the hexoses. It is possible to remove the bitter substance by treatment with carbon. The other products are desirable in that they largely prevent crystallization of the sirup. The most satisfactory sirups have been made by the hydrolysis of 30 per cent lactose solutions.

## PYROLYTIC PRODUCTS

It is a common observation that milk on heating gradually becomes brown, and it is known that lactose is involved in this color formation (145, 157). Ramsey *et al.* (154) claim that caramelization plays no part in the discoloration of dairy products, but that lactose-casein compounds are formed. Kass and Palmer (150) interpret the change as the caramelization of lactose under the influence of casein followed by the adsorption of the lactocaramel on the casein. It is clear that casein promotes the color formation, that it is accelerated in the presence of phosphates and that boric acid and sodium bisulfite, by forming compounds with lactose, hinder the development of color (152, 155). Lactose is the source of the acid, presumably largely formic, that is formed in heated milk (144, 156).

Pyrocatechol has been found in the products of heating a lactose solution to 280° C. under pressure (148). Heating lactose in a solution of sodium hydroxide has yielded a considerable quantity of lactic acid and smaller quantities of formic acid and pyrocatechol (149). Succinic acid has been identified among the products of fusion of lactose with potassium hydroxide (147).

Lactose hydrate loses all its water of hydration below 130° C. The glassy mass formed is very hygroscopic, this characteristic making it very difficult to obtain constant weight in drying dairy products and causing caking of the dried dairy products containing anhydrous lactose. At 150 to 165° C. lactose becomes yellow in color and at 175° C. it becomes brown, emits a characteristic odor and loses about 13 per cent of its original weight (151). This brown material contains anhydrous lactose, a substance insoluble in water, water-soluble lactocaramel and presumably other substances. The caramel may be isolated by first grinding the mass in warm alcohol and removing the lactose by filtration, and then evaporating the filtrate to a sirup, diluting with water and filtering again. This filtrate is evaporated to dryness and the residue dried at 100° C. The final product has a composition corresponding to  $C_{12}H_{20}O_{10}$ . A substance having the same empirical formula has been obtained by Pictet and Egan (153) by dehydrating lactose at 185° C. for 10 to 12 hours under vacuum. They believe it to be lactosan, i.e., galactosyl glucosan. It is insoluble in alcohol and has reducing properties. At 105° C. in the presence of zinc chloride it polymerizes to form a compound having no reducing properties. Hann and Hudson (146) by pyrolytic distillation under vacuum have obtained 6.5 grams each of a d-glucosan and d-galactosan from 100 grams of lactose.

## OXIDATION PRODUCTS

The products obtained by the oxidation of lactose depend on the oxidation potential and concentration of the oxidant used, on the temperature and pH value of the reacting mixture and possibly on other factors.

Potassium permanganate oxidizes lactose in either acid or alkaline solution quantitatively to carbon dioxide and water (175, 176). Oxidation by nitric acid may yield mucic and saccharic acids quantitatively or, if the acid is sufficiently concentrated or hot, it may cause further oxidation to tartaric, racemic, oxalic and carbonic acids (165, 169). One product of the oxidation of lactose by chromic acid is furfural (160). Bromine oxidizes lactose in solution to lactobionic acid. In the presence of a buffer this is the only product, but without buffer, the hydrobromic acid formed causes hydrolysis, the final products being gluconic and galactonic acids (162, 166, 167). Beta lactose is much more rapidly oxidized by bromine than is alpha lactose (168). Iodine reacts with lactose under pressure at 100° C. to give formaldehyde, formic acid and a humic substance containing iodine (182). Neutral hydrated cupric oxide oxidizes lactose to a mixture of formic, lactic and glycollic acids (164).

Hydrogen peroxide has a negligible action on lactose unless an activating substance such as ferrous sulfate is present, in which case lactobionic acid is formed (159, 181). Ozone has no effect on lactose in acid solution; in alkaline solution its action is like that of air (163, 181).

The oxygen of air has no detectable action on lactose solutions unless they are alkaline, in which case it seems probable that salts of lactose are involved (177). In the presence of alkali and such catalysts as cerous hydroxide, ferrous sulfate, sodium sulfite, or sunlight plus zinc oxide as sensitizer, the sugar is oxidized to carbon dioxide and water (179). Without catalytic agents alkali induces auto-oxidation and degradation of lactose, as shown by changes in rotation (158), by the formation of acetic and formic acids (161), and of the saccharinic acids—tetrahydroxycaproic acids—studied extensively by Nef (178) and by Kiliani (170–174). Heating lactose with dilute sulfuric acid causes auto-oxidation to formic acid and beta-acetylpropionic acid (180).

#### HYDROGENATION PRODUCTS

The earliest attempt at hydrogenation of lactose was by Bouchardat (183) who used sodium amalgam and a lactose solution, obtaining a mixture of dulcitol, mannitol (?), sodium lactate, isopropanol, ethanol and hexanol. Neuberg and Marx (187) used calcium amalgam in an atmosphere of carbon dioxide, thus hindering secondary reactions, and obtained a crystalline product that was apparently the lactose alcohol, lactitol, 4-d-sorbitol- $\beta$ -d-galacto-pyranoside, also called lactobiotol and lactositol.

Lactose in aqueous solution was treated by Ipatieff (185) with hydrogen under 74 atmospheres pressure at 130° C. in the presence of a catalyst of nickel and nickel oxide. The only product identified was dulcitol. Senderens (188) and Tanno (189) by substantially the same procedure, except that the catalyst was nickel only, obtained a mixture of lactitol, dulcitol and sor-

bitol. With a hydrogen pressure of only 30 atmospheres and a nickel catalyst, Karrer and Büchi (186) obtained amorphous lactitol and found its specific rotation  $[\alpha]_D = +14.8^\circ$  in water. Wolfson *et al.* (190) treated a slightly acid lactose solution with hydrogen and reduced nickel at a temperature varying from 143 to 150° C. and a pressure from 102 to 138 atmospheres and obtained crystalline lactitol in 80 per cent yield. Their product had a melting point of 146° C. and a specific rotation  $[\alpha]_D^{25} = +14^\circ$  in water. They prepared from it a crystalline tritrityl lactitol hexaacetate, interesting because of its high molecular weight of 1323. Hales (184) has obtained lactitol by electrolytic reduction of an acidified aqueous solution of lactose.

Zartman and Adkins (191) heated lactose, ethanol and hydrogen in the presence of a catalyst of chromium oxide and copper at a temperature of 250° C. and a pressure of 300 atmospheres. This procedure they designated hydrogenolysis, since the product, fractionated under reduced pressure, yielded methanol, more ethanol than used in the process, water, 1, 2-propanediol and three hydroxy compounds tentatively identified as 2-(4-hydroxytetrahydrofuryl)-methyl carbinol, a hexane triol and a hexane tetrol.

#### SUBSTITUTION PRODUCTS

Many more or less complex derivatives of lactose have been described in the chemical journals (14), but, since most of them have little practical interest, they will be mentioned only briefly here, the reader being referred to the cited papers for details of preparation or properties.

Lactose undergoes the characteristic sugar reaction with phenylhydrazine to give first the phenylhydrazone (204) and finally the osazone (203, 231). Several substituted phenylhydrazones of lactose have been prepared (202). Several lactose nitrates have been reported (213), the most definitely authenticated being the octonitrate (243), which has practical value as an explosive (199).

Acetylation of lactose produces heptaacetyl acetyllactoside (200, 217, 219, 238) which, treated with hydrogen bromide, gives heptaacetyl bromolactoside (200, 208). The other halogens may be introduced in a similar manner (195, 206, 216, 229). The reaction of heptaacetyl halogenlactosides with other substances has been used to synthesize other heptaacetyl lactosides (192, 197, 198, 207, 210, 211, 212, 215, 220, 233, 237). Deacetylation converts these compounds to simple lactosides (201, 207, 210, 212, 216, 221, 233). Reduction of heptaacetyl bromolactoside followed by deacetylation yields lactal (193, 209), a substance convertible to an iso and a pseudo modification and to a hydrolactal. Heptamethyl lactosides (26) and heptapropionyl propionyllactoside (222) have been prepared.

Lactose combines directly with amino compounds (223-228, 232, 235, 244). It forms a cyanhydrin which may be converted to lactose carboxylic acid (205, 214, 234). Some other compounds of lactose of possible interest



are the benzoates (230, 240), butyrate (194), butylmercaptan (242), phosphates (241), and its combination with sodium (218), cysteine (236) and calcium carbonate (239).

#### FERMENTATION PRODUCTS

*General discussion.* The products of fermentation of lactose are not peculiar to this sugar, since they are determined by the organism rather than by the substrate and all organisms that ferment lactose ferment other common sugars. On the other hand, many organisms that ferment other common sugars do not ferment lactose. Consequently the chief factors determining whether lactose shall be used as the carbohydrate for a commercial fermentation are the existence of an organism that will convert lactose efficiently into the desired product and the cheapness of whey relative to that of other available sources of fermentable carbohydrate, such as cane, beet or sorghum molasses or corn sugar. The presence of certain vitamins in whey gives it an advantage in some instances.

Of the many substances reported to have been isolated from the products of fermentation of whey, those produced in yield sufficient to warrant consideration for commercial exploitation are ethyl and butyl alcohols, butyric (246, 250, 251), citric (260), acetic (258), propionic (259), and lactic (254) acids, acetylmethylcarbinol (252), riboflavin and penicillin. Lactic acid, penicillin and riboflavin are being produced in this country by fermentation of lactose. The production of ethyl alcohol is possible in the future, but it seems unlikely that the other compounds listed will be made commercially by fermentation of whey.

*Riboflavin.* *Clostridium acetobutylicum*, an organism of the type used to produce butanol and acetone from corn mash, may, by proper culturing, be "trained" to produce riboflavin efficiently from sugar substrates (248, 255, 261). This procedure has been adapted to increasing the riboflavin content of whey to such an extent that the product will contain as high as 1000 micrograms of riboflavin per gram of solids. Details of the process are not yet published.

*Ethyl alcohol.* Although the production of ethyl alcohol from molasses is a firmly established industry and whey is, as a rule, a more costly source of fermentable carbohydrate than is molasses, the production of alcohol from whey in small inland communities with possible further conversion to a whey or spirit vinegar (258) is not economically out of the question. Recently, several articles have appeared (247, 249, 256, 257, 258) and patents have been issued (245, 253), on the production of ethyl alcohol from whey. The techniques described are similar to those for the fermentation of molasses, except that a lactose-fermenting yeast of high fermentative efficiency must be employed instead of the common *Saccharomyces cerevisiae*.

*Lactic acid, manufacture.* Many organisms are able to convert sugars to lactic acid, but for the production of lactic acid from the lactose of whey

a mixed culture of a lactobacillus and a mycoderma is used. This is commonly called "ga" and designated in the American Type Culture Collection as No. 9223. This culture converts lactose to lactic acid with an efficiency of over 95 per cent, the acid being the racemic mixture of the dextro and levo acids, and forms no objectionable byproducts. It grows readily at 43° C. and in a pH range of 5.0 to 5.8, conditions that effectively discourage the growth of organisms that might be present in whey and produce other products, such as, for example, butyric acid (268).

The process in commercial use is substantially that described below (262, 263, 264, 267).

The starter culture is built up by two successive inoculations into increasing quantities of sterilized skim milk and a third inoculation into 500 gallons of pasteurized whey. These cultures are incubated for 24 hours at 43° C. before being used as inoculum for the next larger volume of skim milk or whey.

The fermentation is conducted in a wooden tank of a total capacity of 6000 gallons. The tank should be provided with a perforated steam pipe for heating the whey, a portable agitator for intermittent use and an outlet of adjustable level for decanting.

After the tank is cleaned, chemically sterilized and rinsed free of sterilizer, 5000 gallons of whey are run in and heated to 43° C. The 500 gallons of starter are run in and the fermentation allowed to proceed for about 42 hours, heat being supplied, if necessary, in order to hold the temperature at 43° C. Every six hours, or at shorter intervals if convenient, a slurry of slaked lime is added to neutralize part of the acid and bring the reaction to the most favorable pH range. The lime used should be practically free of magnesia. It should be added gradually with the agitator running until the reaction is at about pH 6.0. At higher pH values there is risk of contamination, and below 5.0 the fermentation is considerably retarded. The pH value may be determined by means of bromocresol green paper or indicator solutions. The completion of the fermentation may be determined by a test for residual sugar with Fehling's solution or judged by the quantity of lime consumed.

The fermented whey is neutralized (pH 6.5–7.5) with lime slurry, heated nearly to the boiling point and held at that temperature for ten minutes or until coagulation of protein is complete. The coagulum is allowed to settle and the clear liquid decanted and run to a filter press, the sludge following. The hot filtrate is pumped to a wooden tank and a small percentage of decolorizing carbon added. The mixture is agitated and brought to a pH value of 10 by the addition of lime slurry, agitation being continued until a sample removed from the batch will sediment rapidly. It is then run to a filter press. The clear filtrate is neutralized by means of lactic acid and, either with or without another carbon treatment, depending on the quality

of product desired, concentrated in a vacuum pan to 15° Bé. The liquid is pumped to crystallizers lined with stainless steel and jacketed for circulating cold water. The mass is cooled to 10° to 15° C., and after about 12 hours standing crystallization is complete.

The crystalline mass is dropped to a centrifuge, the basket spun until no more liquor separates, the crystals washed lightly with water and spun until no more filtrate is obtained. The mother liquor and washings are evaporated to 13.5° Bé. and a second crop of crystals obtained. A third crop may be obtained before it is necessary to discard the liquor and washings.

The wet crude calcium lactate is dissolved in a glass-lined tank in the minimum quantity of water at 65° C., treated with carbon and filter aid, filtered, evaporated to 11.5° Bé., crystallized, centrifuged and washed. This treatment may be repeated, if necessary, in order to produce calcium lactate of U.S.P. grade. The mother liquors and washings are added to the crude lactate liquors.

Calcium lactate solutions of any degree of purity may be converted to lactic acid or lactates of other metals of corresponding grade. Sodium sulfate or carbonate may be used in making sodium lactate, the sulfates of iron and copper in making their lactates. A solution of one of these salts is mixed with a solution of calcium lactate in equivalent proportions, the insoluble calcium carbonate or sulfate removed by filtration and the filtrate concentrated by evaporation or evaporated to dryness.

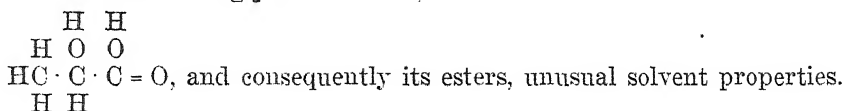
For conversion to lactic acid, sulfuric acid in very slight excess is added to a solution of calcium lactate of approximately 13.5° Bé. in a wooden or ceramic-lined tank. Decolorizing carbon and the calculated amount of potassium ferrocyanide sufficient to precipitate heavy metals present may be added, if acid of one of the better grades is desired. The insoluble calcium sulfate, carbon and ferrocyanides of the heavy metals are removed by filtration through a ceramic vacuum filter. The filtrate, which should contain about 25 per cent lactic acid, may be diluted to 22 per cent and marketed at this concentration or concentrated and subjected to further purification.

Concentration of lactic acid may be accomplished by evaporation in a stainless-steel vacuum pan or, up to over 50 per cent concentration, by a process known as "building up." This latter process consists of dissolving calcium lactate in lactic acid of 25 per cent concentration, made as described above, adding sufficient sulfuric acid to convert the dissolved calcium lactate to lactic acid; filtering, and then adding to the filtrate another increment of calcium lactate and repeating the cycle until the desired concentration of acid is obtained.

Lactic acid is marketed mostly as dark or light, 22 or 44 per cent acid, as colorless, edible 50 per cent acid, and as 85 per cent acid of U.S.P. grade.

*Lactic acid, properties.* The combination of properties that gives lactic acid a somewhat unique position from the standpoint of utility is shared only

by other alpha-hydroxy acids, of which glycollic acid is the only one produced in commercial quantities. The presence of alcohol and acid groups in the same molecule gives lactic acid,



The proximity of the alcoholic and acidic hydroxyls on two adjacent carbon atoms leads to the formation predominantly of linear polylactic esters as water is progressively removed from lactic acid. Inner lactones are not formed under these conditions; the double ester, lactide, is formed in very small proportions and can be prepared only by a slow distillation process involving the dehydration of the dimeric acid. All solutions of lactic acid of concentrations greater than 20 per cent contain linear lactic esters, which increase in both proportion and average chain length with increase in the gross concentration of the acid. As these linear polyesters increase in complexity, they become less susceptible to hydrolytic depolymerization, more viscous and more resinous in character (269, 270, 272, 273, 274, 276).

The heating of calcium lactate forms salts of dilactic acid, which is a result of loss of water from the alcohol groups of two lactyl units to form an ether cross-linkage (275).

*Lactic acid, uses.* The principal uses of lactic acid are in the leather industry, where it is used to neutralize the lime in limed hides (285), in foods, such as pickles, salad dressings, carbonated beverages and sherbets (289, 290, 302, 308, 318, 324) because of its clean acid taste and preservative action, and in alkyd resins (288, 295, 299, 300, 309, 321, 325) because of properties discussed above. Acid calcium lactate is used in Europe in baking powders (280, 287, 297, 298, 305, 306, 307, 315, 327). The ferrous and copper salts are used nutritionally. Sodium lactate solutions have been substituted for glycerol in textile printing and in paper-making (279) and various metal lactates have found use as mordants (296, 312, 326). Many esters of lactic acid have been made for use as solvents and as plasticizers (278, 282, 283, 284, 286, 293, 294, 300, 301, 303, 310, 311, 317, 319, 322, 323). Lactic esters are useful as an addition in molding cellulose ester compositions (277), and as starting materials for the production of the corresponding acrylates (281, 283, 291, 304, 313, 314, 320).

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# OBSERVATIONS ON THE EFFECTS OF AN ANTERIOR PITUITARY PREPARATION ADMINISTERED TO LACTATING DAIRY COWS\*

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In a previous publication Sykes, Meuleman and Huffman (4) reported that both the amount and percentage of fat in the milk of dairy cows were increased with injections of a relatively crude extract of the anterior pituitary gland. The injections were only given for a short period of time (5 days) and no detailed analysis of the milk was made. It was, therefore, thought desirable to give the injections for a longer time and to examine the milk for possible changes in constituents other than fat.

## PROCEDURE

An alkaline extract of the anterior pituitary gland was prepared according to the method of Best and Campbell (1) and used in this study. All injections were given subcutaneously for periods ranging from seven to twenty-three days. Five hundred milligrams of the extract were given at each injection. Four Holstein cows in declining lactation were used in the initial studies, and later the extract was given to an ovariectomized lactating ewe and to a freemartin heifer which had been brought into lactation with hormone treatment.

Daily milk samples were obtained for analysis by mixing equal portions of morning and evening milk. The fat per cent and total solids were determined by the methods of Mojonner and Troy (2). Chlorides were determined by the method of VanSlyke and Sendroy (5) and lactose by the official A.O.A.C. method (3).

## RESULTS AND DISCUSSION

All four cows showed increases in the per cent fat of the milk. The initial increases occurred within three to five days after the first injection. Cow 264 showed an initial increase during the first seven injection days from 4.5 to 5.8 per cent fat, a decline during the next three days to 4.8 per cent and then showed a gradual increase during the remaining thirteen days to attain a level of 10.1 per cent. Two days after injections were stopped this cow developed a severe anemia due to internal hemorrhage and died five days later. Milk production began to decline markedly two days before injections were stopped and the very high terminal fat per cent (10.1) was probably partly due to this fact.

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The milk fat of *Cow A 29* increased from 3.6 to 4.8 per cent on the third day of injection and maintained an average of 4.5 per cent for an additional nine days at which time the fat per cent rapidly fell to 2.0 per cent during the next four days, the udder swelled and ropy and curdy milk was produced. The injections were stopped at this time.

*Cow D 9* increased from a pre-injection milk fat average of 3.9 per cent to an average of 4.3 per cent for a period of nineteen days of injections at which time the per cent fat decreased over a five-day period to 3.5 per cent. Injections were stopped at this point.

The milk fat of *Cow 290* increased from an average of 4.3 per cent to 5.5 per cent on the fifth day of injection. At this point the udder swelled, ropy milk was produced and lactation was terminated three days later at which time the fat per cent had decreased to 4.5 per cent.

It will be noted that the fat content of the milk of three of these cows (*A 29*, *D 9*, and *290*) decreased after an initial increase in spite of continued injections. The fourth cow showed an abnormally high fat content during the last few injection days. Coincident with the drop in fat per cent, the udder of two cows (*A 29*, *290*) became abnormal.

In general, as long as the fat per cent remained within reasonably normal limits and as long as the udders appeared normal, the changes in the other milk constituents tended to parallel the changes in fat with the exception of chlorides which showed either little change or a slight decrease. Moderate increases in solids-not-fat and lactose accompanied the increase in fat. However, when the udders of *A 29* and *290* became abnormal and when the fat content of *264* had increased to about 6.0 per cent very marked changes occurred in the other milk constituents and marked irregularities in their concentration occurred from day to day in three of the four cows. The fat, solids-not-fat and lactose decreased markedly and then tended to return to normal levels when injections were stopped. All the constituents and particularly the lactose varied considerably from day to day, a condition which persisted for nearly a month after injections were terminated. The chloride concentration was generally inversely proportional to the lactose level. *D 9* did not react in this manner and, in fact, did not respond as well in respect to increase in fat as did the other cows. This cow also differed from the other three in that she was not pregnant.

The level of production in all cows remained equal to or was slightly above the pre-injection level during the time the initial fat increases occurred, but declined rapidly when the udders became abnormal. The changes which occurred in the milk of *A 29* are generally typical of the changes which occurred in the group as a whole. These are presented in figure 1.

In addition to the above changes it was found that all four cows developed cystic ovaries. Three cows developed multiple cysts of both ovaries and the fourth cow developed cysts on the right ovary. None of the three



pregnant cows terminated a normal pregnancy. Mummified fetuses were removed from two of the cows. The third cow (264) died, as mentioned previously, and it is not possible to state definitely whether mummification of the fetus had commenced although there were indications that such was the case.

These latter observations suggested that the increase in fat percentage and other changes in the milk of these cows was possibly due to the intense ovarian stimulus which the extract produced. Since the extract is known to possess gonadotropic hormone and since ovarian effects accompanied the changes in fat content of the milk, it seemed possible that this hormone might

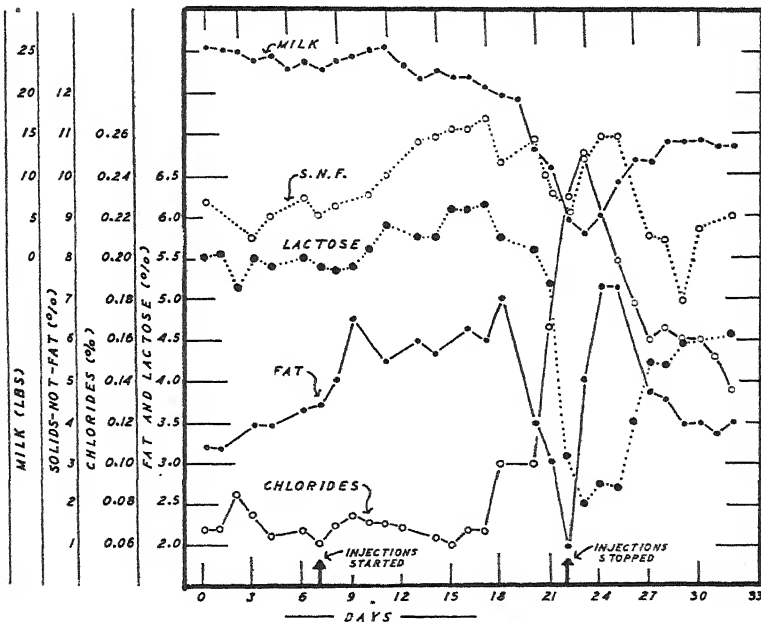


Fig. 1. Changes in milk constituents following injections of an anterior pituitary extract.

indirectly affect fat metabolism through ovarian stimulation. If such were the case it would not be necessary to postulate the existence of a separate fat metabolism hormone of the pituitary gland at least insofar as such a hormone affects the fat content of milk.

The extract was therefore administered to a lactating ewe (250 mg. daily) which had been ovariectomized two weeks previous to the first injection and to a freemartin heifer (500 mg. daily) which had been brought into lactation by prolonged treatment with estrogens and progesterone followed by prolactin. At autopsy the ovaries of this heifer were practically non-existent and showed no signs of activity whatsoever.

The fat content of the milk of these two animals increased as a result of the injection of the extract in a manner similar to the cows. These results are shown in table 1. While the data are not extensive they suggest that the fat increase in the milk of cattle injected with this extract of the pituitary was not due to the ovarian stimulation. Unpublished results also indicate that the increase in liver fat which the extract produces in guinea pigs occurs just as readily in ovariectomized as in normal pigs. While it is possible that the gonadotropic hormones may have some direct effects on fat metabolism not mediated through the ovaries, there is at present no evidence

TABLE 1

*The effect of an anterior pituitary extract on the per cent fat of the milk of an ovariectomized ewe and a freemartin heifer*

Day	Fat content of milk		
	Ovariectomized ewe	Freemartin heifer	
	<i>per cent</i>	<i>per cent</i>	
1	9.5	3.7	No injections
2	8.6	3.9	"
3	10.0	3.4	"
4	8.0	3.0	"
5	8.7	.....	Injection
6	9.7	3.7	"
7	10.5	.....	"
8	10.7	3.7	"
9	10.5	3.4	"
10	15.0	3.6	"
11	11.7	3.6	No injections
12	15.3	4.4	"
13	19.5	4.3	"
14	15.5	3.6	"
15	12.5	4.0	"
16	12.5	.....	"
17	8.5	4.4	"
18	6.8	.....	"
19	.....	4.2	"

for this and the effects on fat metabolism produced by the extract used in these experiments would seem to be due to some other hormone, possibly a separate fat metabolism hormone.

#### SUMMARY AND CONCLUSIONS

Injection of an alkaline extract of the anterior pituitary gland into lactating cows produced an initial increase in the per cent fat of the milk and moderate increases in lactose and solids-not-fat. The chloride content was generally inversely proportional to the lactose level.

Continued injections produced even more marked changes. Generally, the fat, lactose and solids-not-fat declined to very low levels and the chloride increased. Marked irregularities occurred from day to day. These latter effects have not been observed to occur earlier than the fifth day of injection

and in one animal injections given for twenty-four days failed to produce them. Swelling of the udder andropy milk accompanied these changes.

The extract likewise produced cystic ovaries in all the cows to which it was given and mummified fetuses were removed from two of the three pregnant animals used following the injection period.

Preliminary work indicates that the changes in fat percentage of the milk were not due to the ovarian stimulation which was produced by the extract and it would appear that these changes were due to some hormone other than the gonadotropins contained in the extract.

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# THE INFLUENCE OF A SYNTHETIC THYROPROTEIN WHEN FED TO DAIRY COWS OVER A THREE-WEEK PERIOD\*

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## INTRODUCTION

Graham (5) was the first to observe that the thyroid gland has a profound influence on the level of milk and milk-fat secretion of cows. He (6) later identified the active principle of the thyroid gland as thyroxine. These observations have since been confirmed by a number of investigators (7, 8, 1, 2, 3, 14, 9, 4).

Although the thyroxine studies added much to our knowledge of the factors controlling the level of milk and milk-fat secretion, no practical application of the results was possible because of the excessive cost of thyroxine. Recently Reineke and Turner (13) have made it more practicable by the formation in vitro of highly active thyroproteins. Reports on the effect of feeding such thyroproteins to dairy cows have also appeared (12, 10, 13, 11).

This work was begun to determine whether or not it is possible to increase the fat content of milk, without greatly augmenting milk production, by feeding a moderate daily dose of thyroprotein.

## EXPERIMENTAL PROCEDURE

Five dairy cows in varying stages of declining lactation and gestation were selected for the experiment (table 1). Available production records at the beginning of the experiment indicated that the cows were secreting milk with a fat content below that of their respective breed averages.

TABLE 1

*Description of cows fed a synthetic thyroprotein for a three-week period*

Cow No.	Breed	Age Yr.—Mo.	No. of lactation	Month of lactation	Month of gestation
H-41	Holstein-Friesian	3	1st	10th	7th
H-37	"	4—11	2nd	8th	3rd
627	Brown Swiss	3—5	1st	5th	1st
492	Ayrshire	3—5	1st	6th	5th
383	Jersey	3—1	1st	4th	0

The experiment consisted of a two-week pre-experimental period, a three-week experimental period, and a two-week post-experimental period. Ten

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grams of a thyroprotein (Protamone<sup>1</sup>) were fed daily in the grain ration during the three-week experimental period. During the entire course of the experiment daily milk weights (twice daily milkings) were recorded and milk samples were taken on two consecutive days each week. Individual milk samples were tested for their butterfat content (Babcock method) and solids-not-fat content (by means of a lactometer). Body weights and heart rates (by means of a stethoscope) were determined on two consecutive days each week.

#### EXPERIMENTAL RESULTS

*Fat test.* The fat test, on the average, increased from 3.62 to 4.11 during the feeding period and then declined to 3.76 in the second week of the post-experimental period. Increases in fat test during the course of the entire experiment varied from 0.34 to 1.51, the latter increase being observed in the cow most advanced in lactation. It is interesting to note that the withdrawal of Protamone from the ration of a cow (H-41) advanced in lactation, as well as cows not so far advanced in lactation, resulted in a decrease in fat test. The decrease in fat test in the second week of the post-experimental period is indeed remarkable since it occurs at a time when milk production is decreasing. The results are summarized in table 2.

TABLE 2

*Influence of feeding 10 grams of Protamone daily on the fat content of milk*

Period	No. of days	Cow number					Weighted ave.
		H-41	H-37	627	492	383	
		% fat	% fat	% fat	% fat	% fat	% fat
Pre-experimental	14	3.31	3.03	3.79	3.29	4.80	3.62
Experimental	7	3.24	3.08	3.96	3.15	4.58	3.61
	7	3.63	3.30	4.15	3.68	4.88	3.90
	7	3.58	3.37	4.25	4.25	5.23	4.11
Post-experimental	7	4.82	3.27	4.17	3.87	5.25	4.13
	7	3.67	3.10	3.90	3.57	5.02	3.76
Maximum increase during experiment	.....	1.51	0.34	0.46	0.96	0.45	0.51

*Milk production.* Protamone feeding stimulated varying increases in milk production. Based on weekly averages, the maximum increase in milk production was 1.7 lbs. per day. Increases in individual cows (weekly averages) varied from 0.5 to 4.7 lbs. Two cows (H-41 and 383) attained their highest average daily production during the first week of the feeding period while two other cows (H-37 and 627) did not reach their highest average

<sup>1</sup> The Protamone was generously supplied by the Cerophyl Laboratories, Kansas City, Missouri, through the courtesy of Dr. W. R. Graham, Jr.

daily production until the third week of the feeding period. The cow (H-37) showing the greatest increase in milk production showed the least increase in the fat content of her milk and conversely the cow (H-41) giving the smallest increase in milk production showed the greatest increase in the fat content of her milk, the latter being most advanced in her lactation period.

On the removal of Protamone from the ration there was a slight decrease in milk production. Nevertheless the level of milk production during the post-experimental period appeared to be about the same as it would have been had Protamone not been fed (table 3).

TABLE 3  
*Influence of feeding 10 grams of Protamone daily on milk production*

Period	No. of days	Cow number					Ave.
		H-41	H-37	627	492	383	
		<i>lbs. milk per day</i>	<i>lbs. milk per day</i>	<i>lbs. milk per day</i>	<i>lbs. milk per day</i>	<i>lbs. milk per day</i>	<i>lbs. milk per day</i>
Pre-experimental	14	15.2	29.3	26.1	23.5	22.3	23.3
Experimental	7	15.7	31.7	25.8	25.6	25.6	24.9
	7	15.3	33.1	27.7	25.8	23.2	25.0
	7	14.6	34.0	28.4	24.9	22.9	25.0
Post-experimental	7	13.0	31.2	25.2	23.0	20.3	22.5
	7	10.3	29.0	23.3	21.1	16.8	20.1
Maximum increase during experiment (weekly average)	.....	0.5	4.7	2.3	2.3	3.2	1.7

*Solids-not-fat.* The solids-not-fat content of the milk did not appear to be affected by Protamone feeding. The five cows showed an increase in the solids-not-fat content of their milk during the experimental period but it is doubtful if this increase can be attributed to the feeding of Protamone since only 2 of the 5 cows showed a decrease in the solids-not-fat content of their milk in the post-experimental period. The average figures suggest that the observed increase in solids-not-fat content can be attributed to the advance in the stage of lactation (table 4).

*Body weight.* There was a slight loss in body weight in four of the five cows. The cow that did not lose in body weight was the one (H-41) most advanced in lactation and pregnancy. The greatest loss in body weight occurred in the lightest cow of the group and this would be expected since Protamone was not fed on the basis of body weight. In no instance, however, can the loss in body weight be considered severe.

There was a marked increase in body weight in the first week of the post-

experimental period. This gain in body weight is associated with a decrease in milk production and in heart rate. In the second week of the post-experimental period four of the five cows showed a slight loss in body weight and this loss in body weight was associated with an increase in heart rate. The cow (383) that continued to show a gain in body weight during the second

TABLE 4

*Influence of feeding 10 grams of Protamone daily on the solids-not-fat content of milk*

Period	No. of days	Cow number					Weighted Ave.
		H-41	H-37	627	492	383	
		% S-N-F	% S-N-F	% S-N-F	% S-N-F	% S-N-F	% S-N-F
Pre-experimental	14	8.32	8.13	9.13	8.87	9.24	8.74
Experimental	7	8.49	8.19	9.14	8.90	9.30	8.80
	7	8.83	8.29	9.14	9.16	9.40	8.96
	7	8.73	8.36	9.38	8.94	9.15	8.91
Post-experimental	7	8.97	8.27	9.28	9.14	9.55	9.04
	7	8.95	8.40	9.35	9.11	9.59	9.08

week of the post-experimental period was the one that had lost the most in body weight during the experimental period and the one that showed the greatest decrease in milk production during the post-experimental period. The body weight averages are presented in table 5.

TABLE 5

*Influence of feeding 10 grams of Protamone daily on body weight*

Period	No. of days	Cow number					Ave.
		H-41	H-37	627	492	383	
		<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
Pre-experimental	14	1170	1219	1210	1081	791	1094
Experimental	7	1187	1204	1219	1100	777	1098
	7	1192	1242	1208	1086	781	1102
	7	1178	1212	1189	1077	755	1082
Post-experimental	7	1245	1310	1272	1138	781	1149
	7	1241	1279	1240	1119	816	1139
Maximum decrease in body weight	.....	.....	15	21	4	36	12

*Heart rate.* At the end of the second week of the experimental period heart rate had increased from 74 beats per minute to 83 beats per minute. All of the cows showed increases in heart rate, with the increases varying from 8 to 14 beats per minute. The cow (492) with the lowest initial heart



rate had the greatest increase in heart rate (14 beats per minute) while the cow with the highest initial heart rate (H-37) showed the least increase in heart rate (8 beats per minute).

In the third week of the feeding period heart rate was similar to that of the pre-experimental period. This decrease in heart rate cannot be accounted for, but it was not caused by temperature changes. That the Protamone was stimulating heart rate during the third week of the experimental period is attested by the fact that there was a further decrease in heart rate during the post-experimental period (table 6).

TABLE 6  
*Influence of feeding 10 grams of Protamone daily on heart rate*

Period	No. of days	Cow number					Ave.
		H-41	H-37	627	402	383	
		<i>Rate per minute</i>	<i>Rate per minute</i>	<i>Rate per minute</i>	<i>Rate per minute</i>	<i>Rate per minute</i>	<i>Rate per minute</i>
Pre-experimental	14	71	82	76	68	74	74
Experimental	7	72	84	88	80	85	82
	7	83	90	77	82	84	83
	7	73	75	71	71	76	73
Post-experimental	7	70	64	58	64	55	62
	7	72	70	60	68	76	69
Maximum increase in heart rate	.....	12	8	12	14	11	9

#### DISCUSSION

It appears that the feeding of Protamone in a moderate daily dose will definitely increase the butterfat content of milk. This increase in butterfat content is not accompanied by either any great increase in milk production or a great loss in body weight. There are no objections to increases in milk production; in fact, they are most desirable, provided they can be obtained without encountering severe losses in body weight. Certain cows are good milk producers but low testers. Obviously such cows should not be fed large amounts of Protamone, for that would undoubtedly result in severe losses in body weight. If, however, moderate doses of Protamone will increase fat test, and this experiment indicates that they will, then it is certainly advantageous to feed Protamone. On the other hand, many cows increase in body weight at the expense of milk production, and such cows should receive larger doses of Protamone.

#### SUMMARY

The feeding of 10 grams of Protamone daily for 3 weeks to a group of 5 dairy cows increased the butterfat content of the milk from 3.62 per cent to 4.11 per cent. The average milk production was increased from 23.3 lbs. to

25.0 lbs. per day. Losses in body weight were slight and heart rate increases were moderate. Solids-not-fat did not appear to be affected.

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# A CONTROLLED EXPERIMENT IN FEEDING WHEAT GERM OIL AS A SUPPLEMENT TO THE NORMAL RATION OF BULLS USED FOR ARTIFICIAL INSEMINATION<sup>1</sup>

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Though the importance of adequate vitamin E in the rations of rats for normal reproduction is well known (7), no clear-cut evidence has been obtained that the reproductive performance of the larger farm animals is affected by insufficient vitamin E. In 1931 Vogt-Moller and Bay (18) reported that injections of wheat germ oil at the time of heat, or just before, were effective in curing sterility in some cows, which had failed to settle on repeated earlier services, but which otherwise appeared normal. Later (2) these same investigators reported continued success with their uncontrolled treatments. In controlled studies with a limited number of cows Asdell *et al.* (1) were unable to show that injections of wheat germ oil gave results different than those obtained with untreated controls. Gwatkin and MacLeod (4) showed that wheat germ oil therapy was ineffective in altering the course of *Brucella abortus* infection in a group of 12 cows compared with a similar group of controls.

Thomas and collaborators (14, 15, 16, 17) in extensive studies with goats and sheep have shown that in these species, at least, the vitamin E requirements are either very low or that a dietary supply of this vitamin is not needed. In these studies the vitamin E in the basal diet was destroyed by treatment with an ether solution of ferric chloride and aging. Normal reproduction of the goats and sheep proceeded on this ration which was so low in vitamin E that rats were unable to reproduce on it.

Titus and Burrows (13) have determined the effect on semen production of 0.5 per cent of wheat germ oil in experimental rations of cockerels. They found that wheat germ oil decreased semen production. No similar study with the larger farm animals has come to the attention of the writer.

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<sup>1</sup> The investigation was conducted in cooperation with the New York Artificial Breeders' Cooperative, Inc., Syracuse, N. Y., who placed their bulls and other facilities at the disposal of the author, and in cooperation with the Viobin Corporation, Monticello, Ill., who supplied the wheat germ oil used. The cooperation of these parties is sincerely appreciated.

<sup>2</sup> The author wishes to acknowledge his indebtedness to Dr. Karl E. Mason, Department of Anatomy, University of Rochester Medical School, Rochester, N. Y., for conducting the bio-assays of the vitamin E potency of the feeds used, to his colleagues, Professors J. K. Loosli, L. A. Maynard and F. B. Morrison, for aid in planning the investigation and in the preparation of the manuscript, to Drs. P. T. Cupps and F. Irvine Elliott, for collecting certain portions of the data, and to Maurice Johnson and Harold Rosa, for their aid in conducting the experiment.

Hathaway, Davis and Graves (5), Hathaway and Davis (6), and Palmer, Nelson and Gullickson (8) have determined in bio-assays with rats the vitamin E content of a number of the feeds commonly fed to dairy cattle. Their results indicate that most rations for dairy animals, composed of natural, common feeds and satisfactory in other respects, would contain liberal amounts of vitamin E for satisfactory reproduction in rats. This fact and the results of the other investigations cited raise the question of the value of supplementing a practical ration composed of natural feeds with wheat germ oil as a source of vitamin E to prevent sterility in dairy animals. In spite of these facts, the use of wheat germ oil as a supplement to the normal rations of breeding farm livestock, especially males, is quite widespread to judge from verbal reports received from livestock men.

In view of these facts it was considered desirable to determine the value of supplementing a normal ration composed of usual, common feeds for dairy bulls with wheat germ oil over a sufficiently long period of time to determine whether or not the added wheat germ oil had an effect on semen production and upon the fertility of that semen when used for artificial insemination.

#### EXPERIMENTAL

Twenty bulls were selected for the investigation from the 28 bulls then owned by the New York Artificial Breeders' Cooperative and housed in one barn near Syracuse, New York. These bulls were of the Holstein-Friesian and Guernsey breeds and were used only in artificial breeding. The 20 bulls were divided into two groups as nearly equal in all respects as could be determined. The basis of classifying the bulls into groups is given in table 1. This table gives the mean for each bull, the mean of the group and the standard deviation of the group means for each criterion used in the grouping. The relative fertility of the bulls was established before the experiment started by the proportion of the cows to which each bull was artificially bred during a three months' period, which did not return to service for at least one month after being bred. The data on average semen volume and average motility of the spermatozoa for each bull was obtained during the same period of time. On the other hand, the spermatozoa counts (table 1) are for but one collection taken during the week before the experiment started, and are less reliable than the other measures. The weight of each bull was estimated from heart girth measurements.

As can be seen from the table the two groups were very similar in the criteria used for classification. After the individual groups were selected a flip of a coin by a disinterested party determined that Group I would receive the wheat germ oil. This group of bulls was called the wheat germ oil-fed group, while Group II was known as the control group.

All bulls were fed the same concentrates and hay twice daily. Hay was the only roughage fed. It was composed of about 50 per cent alfalfa, 40 per

TABLE 1  
Criteria for grouping bulls

Bull number	Breed*	Age	Estimated weight	Average volume semen	Spermatozoa count per mm. <sup>3</sup>	Average motility	Services	Non-returns to service
Wheat germ oil-fed group (I)								
		mo.	lbs.	ml.	thousands	%		%
1E	H	40	1700	5.8	772	72	150	58.0
2E	H	109	2125	7.6	897	73	269	63.2
3E	H	123	2025	6.7	623	70	119	64.7
4E	H	20	1197	3.5	1,178	74	113	77.9
5E	H	90	1791	5.1	963	66	78	76.9
6E	H	51	2075	5.4	1,783	77	100	62.0
7E	H	13	853	2.0	1,377	60	28	57.1
8E	G	87	1745	7.1	718	70	144	64.6
9E	G	86	1791	8.1	1,412	78	149	65.8
10E	G	97	1538	9.0	1,945	80	98	70.4
Mean	.....	71.6 ± 38.0	1684 ± 400	6.03 ± 2.14	1,166.8 ± 454.2	72.0 ± 5.94	124.8 ± 62.8	66.1 ± 7.1
Control group (II)								
1C	H	37	1607	5.1	1,387	73	105	58.1
2C	H	98	2205	9.0	700	74	205	64.4
3C	H	121	1837	5.1	1,443	76	155	65.8
4C	H	25	1377	2.5	987	68	55	61.5
5C	H	78	2025	4.9	1,507	72	107	76.6
6C	H	66	2125	6.3	1,777	70	18	61.1
7C	G	17	908	2.9	1,123	66	107	66.4
8C	G	78	1607	7.2	703	81	112	55.4
9C	G	52	1630	7.7	803	68	131	71.8
10C	G	96	1607	7.5	1,129	76	68	60.3
Mean	.....	66.8 ± 33.9	1693 ± 383	5.82 ± 2.11	1,155.9 ± 367.1	72.4 ± 4.57	106.3 ± 53.4	64.2 ± 6.4

\* H = Holstein-Friesian.

G = Guernsey.

cent or less of timothy, and about 10 per cent other grasses and clover. The hay varied somewhat throughout the experiment, but was largely of No. 2 grade. When the experiment was first started, April 15, 1941, hay grown the previous season was fed. After July 15, hay produced during that season was fed, and was continued to the end of the experiment, April 14, 1942.

The concentrate mixture was the one the Cooperative had been feeding for some time. It was made up of normal feeds ordinarily available to any dairyman and contained the following proportions of ingredients:

46.5 lbs. ground oats
18.5 lbs. yellow cornmeal
19.0 lbs. wheat bran
4.5 lbs. linseed meal
4.5 lbs. soybean oil meal
4.5 lbs. steamed bone meal
2.5 lbs. salt
<hr/>
100.0

The mixture contained approximately 14 per cent total protein. The feed allowance of both concentrates and hay was weighed one day each week throughout the experiment. On this basis, the bulls in Group I received an average estimated daily allowance of 20.6 pounds of hay and 7.7 pounds of the concentrate mixture. The control group (II) received an estimated average of 20.8 pounds of the hay and 8.0 pounds of the concentrate mixture daily.

Three samples each of the hay and concentrate mixture were assayed with rats at different times during the experiment for vitamin E potency by Dr. Karl E. Mason. Female rats, critically depleted of vitamin E at the time of weaning, were subsequently reared upon an E-deficient diet modified by substituting a variable proportion of the test samples for a corresponding portion of the cornstarch component. The success or failure of their first pregnancy was used as a basis for determining the adequacy or inadequacy of the modified diet. With the first and third hay sample complete fertility response resulted when the hay made up but 20 per cent of the modified diet. For the second hay sample, 25 per cent was needed, and for each of the three samples of the concentrate mixture 30 per cent of the modified diet was necessary for complete protection.

One ounce of solvent process wheat germ oil<sup>3</sup> daily was fed to each bull in Group I. The oil was poured on the concentrate mixture at each night feeding. The oil was weighed out to each bull from one quart containers which were kept in the ice-box until completely used. A fresh supply of the oil was received at approximately one-month intervals.

<sup>3</sup> The Viobin Corporation report that the oil contained approximately 2 Evans rat units of vitamin E per gram.

The first nine bulls of both groups were started on the experiment on April 15. On June 1, one bull was added to each of the experimental groups. These are listed as bulls 10E and 10C in table 1. The experiment was continued through April 14th of the following year. Thus, nine bulls of each group were on the experiment from the beginning and, if they continued to produce semen of satisfactory quality, were expected to remain on the rations for one year. For the remaining bull in each group the planned duration of the experiment was for ten and one-half months.

Semen was collected from each bull in the experiment approximately once each week. Ordinarily the bull was sampled at each of these collection periods as many times as was necessary to obtain the required quantities of semen of high enough quality to give satisfactory assurance that the expected number of cows in heat could be impregnated by it. Often two ejaculations at a collection period were taken and sometimes three or more were collected from a single bull in one day. The collection of this many ejaculates from a bull in one day was due usually to the fact that the first collection of semen was so low in spermatozoa count or so low in spermatozoa motility that it was discarded. Each semen sample which, by the criteria used, gave indications of being satisfactory for artificial insemination, was diluted with the yolk-citrate diluent (10) and shipped to the various sections of New York State where it was used for inseminating cows. The dilution rates for the semen used in this experiment were similar for each group of bulls and varied from 1 part of semen to 2 parts of the diluter up to 1 part of semen to 16 parts of the diluter. The semen was diluted at rates largely dependent upon the number and activity of the spermatozoa in each sample. Salisbury *et al.* (11) have shown that similar rates of conception may be expected for semen diluted on this basis and within the range of dilution used in this investigation. One ml. of diluted semen was used for each insemination. The spermatozoa counts were made with the hemocytometer. The methods used to determine motility and in handling the semen before insemination were described earlier (19).

## RESULTS

The results of the investigation are presented in two parts; the first deals with the breeding behavior of the bulls and the characteristics of the semen produced by them; the second deals with the fertility of the two groups of bulls when the semen was used for artificial insemination.

*Breeding behavior of the bulls and semen characteristics.* Table 2 presents the mean data relative to the semen characteristics measured of each group of bulls. These measurements were made throughout the entire experimental period. The time required for service was determined only during the first six months of the experiment and the mean time, also, is shown in table 2. This, so-called, "service time" is the number of seconds from the time the bull approached a fixed point about 4 feet behind the

"teaser" cow to the time of ejaculation. When more than one ejaculate was taken at the time of collection, the bull was removed some ten feet from the cow until preparations were completed for the second collection. This time interval varied from about 3 to 5 minutes.

In many cases, the semen by gross observation or as a result of the microscopic examination or spermatozoa count, was considered unfit for use in artificial insemination. Such samples were discarded and are so listed in table 2. In other cases it was found impossible for one reason or another

TABLE 2

*Characteristics of semen produced by experimental bulls and their breeding behavior*

	Ejaculate number	Wheat germ oil-fed group (I)	Control group (II)
Total number of ejaculates collected	1	335	335
	2	219	281
	3	38	62
	4	2	4
	All	594	682
Average volume of ejaculates, cc.	1	5.9 (312)	5.3 (301)
	2	6.4 (203)	6.6 (253)
	3	5.2 (34)	5.5 (55)
	All	6.0 (570)	5.7 (654)
Average motility of spermatozoa in ejaculates, per cent	1	71.0 (282)	69.8 (261)
	2	72.7 (184)	76.6 (231)
	3	72.2 (27)	74.9 (50)
	All	71.4 (533)	73.0 (606)
Average number of spermatozoa in ejaculate, 1,000's per mm. <sup>3</sup>	1	1,323 (179)	1,259 (160)
	2	1,113 (115)	1,281 (170)
	3	1,099 (15)	1,008 (28)
	All	1,207 (409)	1,227 (450)
Average service time, seconds	1	193 (159)	108 (147)
	2	166 (98)	124 (120)
	3	105 (17)	78 (27)
	All	174 (302)	104 (353)
Total volume semen discarded, cc.	...	652 (120)	620 (123)
Ejaculates discarded, volume unknown	.....	( 15)	( 25)

to obtain all of the information desired on each semen ejaculation. Occasionally, two successive ejaculates were taken in the same artificial vagina, without using two different collection tubes. The average data for such combined ejaculates are included in table 2 under the totals and explain the discrepancy found in the table for the number of samples which were used to obtain the average for all ejaculates and the sums of the number of ejaculates used for the average of the first, second and third ejaculates. Fourth ejaculates were so few in number that they are included in the average for all ejaculates only. In this table the data are an average of those samples for which observations were made and the number of ejaculates on which each average is based is included in parenthesis after the data. There was



no apparent difference between the experimental groups in this regard. From this fact it is believed that the semen produced by each group of bulls has been well sampled and that conclusions may be drawn from this table concerning the effects of the experimental treatments.

More individual ejaculates of semen were collected from the control bulls than was the case with the wheat germ oil-fed bulls. This was due primarily to the fact that the average volume of the first ejaculates, of which an identical number was collected from each group of bulls, was enough smaller for the control bulls so that, in order to have sufficient semen for breeding, it was more often necessary to collect a second ejaculate. This result was not surprising for the bulls in Group II produced a smaller quantity of semen in the first ejaculates during the three months preliminary period before the experiment started. In fact the mean volume of all ejaculates for the experimental period varied little from the mean volume of all ejaculates obtained during the preliminary period. In addition, there was a greater demand for the semen of these bulls as indicated by the fact that they were bred to 884 more cows during the experiment than were the bulls of Group I.

To test this apparent difference in semen volume statistically and to determine whether or not there were other differences between the experimental groups, the mean data for all ejaculates of each bull were arranged by months and an analysis of variance made. In each case the accepted method of analysis when unequal numbers are found in the groups was used (12). For semen volume, per cent of motile spermatozoa, spermatozoa count and number of discarded ejaculates there were no statistically significant differences between the wheat germ oil-fed and the control groups.

With respect to the time required for service, as a measure of the sex drive of the bulls, there was a highly significant difference in favor of the control bulls. The difference in actual time, however, was only slightly more than one minute and was not of great practical importance. Two bulls on each ration, Nos. 6E, 8E, 8C and 9C, were discarded from the experiment for failure to satisfactorily settle the cows to which they were artificially bred or for refusal to use the artificial vagina. One other bull on each of the rations was not used for various lengths of time during the experiment because of injuries which had nothing to do with the rations fed. Table 3 gives the pertinent data on the bulls which were removed from the experiment. The bull 10E which was not used from July 10 to November 1, because of lameness, was fed the experimental ration throughout this period. The other bull which was injured, 4C, did not recover from the badly bruised knee and was sold.

From the fertility data obtained before the start of the investigation, bulls 6E and 8E, though slightly lower in per cent of non-returns to service than the mean of Group I, were not expected to drop in fertility. After the

first few months on the experiment it was apparent that they were decreasing in fertility. For best field results in artificial insemination it would have been desirable to use them no further for breeding. However, it was desired to continue the wheat germ oil feeding over a sufficiently long period of time to determine whether they could be improved in fertility. When no improvement resulted, after 8 months in the case of one bull and 10 months with the other, they were removed from the experiment. They were both used for breeding during this period.

Bull 8C was the lowest in fertility of the control bulls during the preliminary period, though not so low that he was not expected to continue in service. This bull was used regularly for three and one-half months and so many of the cows to which he was artificially bred failed to settle that the

TABLE 3  
*Bulls removed from the experiment and reasons for removal*

Bull number	Dates used	Reasons for removal	Spermatozoa count per mm. <sup>3</sup>	Motility	Fertility to date†		
					S	C	%
Wheat germ oil-fed bulls							
6E	4/15/41 to 2/21/42	Low fertility	<i>thousands</i> 1,332 (35)*	74 (46)	364	143	39.3
8E	4/15/41 to 12/11/41	Low fertility	928 (27)	71 (34)	219	73	33.3
10E	6/ 1/41 to 7/10/41 and 11/ 1/41 to 4/14/42	Lameness— rested for about 3½ months					
Control bulls							
4C	4/15/41 to 7/14/41	Bruised knee—sold for beef					
8C	4/15/41 to 4/ 4/42	Low fertility	752 (14)	57 (17)	61	24	39.3
9C	4/15/41 to 10/29/41	Refusal to use artificial vagina	1,970 (10)	67 (12)	125	65	52.0

\* ( ) = ejaculates in average.

† S = services. C = conceptions.

semen was not used for insemination after July 30. Semen samples were collected on November 25, 1941, March 26 and April 4, 1942, but all were of such poor quality that they were not used for breeding cows. Bull 9C settled as many cows as the average bull in either group during the 6 months he remained in the experiment. However, he became reluctant to use the artificial vagina and finally refused to mount the teaser cow. He was kept on hand until February, 1942, but could not be induced to attempt to mount. It is believed that an injury was responsible for this condition.

*Effect of season on semen characteristics.* In table 4 are shown the average volume, spermatozoa count and per cent of motile spermatozoa in the

semen collected from each group of bulls arranged by months. These data show a tendency for the per cent of motile spermatozoa to be somewhat lower in the early spring months, and the concentration of spermatozoa to be lowest in August. No significant difference between months was found for service time, and for volume of semen, but for spermatozoa count and per cent of motile spermatozoa the differences between months were highly significant ( $< 1.0$  per cent level of probability). For the number of ejaculates discarded the difference between months was just significant at the 5 per cent level of probability. These data indicate that the seasonal fluctuations in semen quality which occurred in central New York with the 20 bulls were not as great as those reported for 4 bulls in Indiana by Erb, Andrews, and Hilton (3), or for 6 bulls in Maryland by Phillips *et al.* (9).

*Fertility of the bulls.* The method used to determine the fertility of the bulls is presented in detail elsewhere (11). In brief, it consisted of deter-

TABLE 4

*Effect of season on semen characteristics. Average of all ejaculates for which information was available*

	Wheat germ oil-fed group			Control group		
	Volume	Concentration per mm. <sup>3</sup>	Motility	Volume	Concentration per mm. <sup>3</sup>	Motility
	cc.	thousands	%	cc.	thousands	%
April 15-30 .....	6.3 (25)	.....	63.5 (23)	5.1 (31)	.....	59.3 (30)
May .....	5.8 (53)	1120 (48)	64.9 (51)	5.3 (58)	1094 (58)	65.4 (57)
June .....	6.2 (57)	1348 (49)	72.5 (54)	5.2 (62)	1436 (54)	73.0 (61)
July .....	6.2 (50)	1133 (50)	73.6 (54)	5.3 (69)	1128 (62)	77.0 (69)
August .....	5.6 (58)	977 (53)	76.9 (58)	5.6 (56)	1055 (46)	75.1 (54)
September .....	6.0 (48)	1078 (35)	76.0 (45)	5.9 (56)	1198 (51)	79.8 (55)
October .....	5.5 (28)	1338 (24)	73.1 (29)	5.6 (48)	1511 (36)	78.6 (46)
November .....	6.3 (52)	1247 (28)	71.2 (42)	6.3 (39)	1211 (22)	71.7 (33)
December .....	5.9 (50)	1241 (29)	69.8 (48)	5.5 (57)	1252 (33)	71.8 (53)
January .....	5.4 (48)	1287 (25)	76.0 (43)	6.4 (46)	1216 (30)	76.1 (46)
February .....	6.1 (41)	1474 (25)	67.6 (35)	5.8 (54)	1417 (23)	69.2 (51)
March .....	5.9 (44)	1312 (28)	66.9 (36)	6.0 (59)	1185 (49)	72.8 (43)
April 1-14 .....	6.4 (16)	1260 (15)	68.7 (15)	6.3 (19)	1182 (16)	70.0 (8)

mining the proportion of the cows to which each bull was bred which did not return to service within a period of at least five months after service. Such cows were considered to be pregnant. This method varied from that employed to determine the relative fertility of the bulls during the preliminary period for, in this case, information regarding fertility was desired immediately prior to the start of the experiment and it was impossible to wait until the five months' period had elapsed.

The summarized data covering the entire experimental period is presented in table 5. In the original data the number of services and conceptions were summarized for each bull for each month. The percentage of the total services resulting in conception were then calculated and an unweighted

analysis of variance of the percentage figures, using the accepted methods for analysis when unequal numbers are found in the groups (12), was made. In a previous publication (11) it was felt necessary to use a weighted analysis because the per cent of services resulting in conception from individual ejaculates was under consideration. In this case the data for each month are a summary of several ejaculates and the extremely wide range in services per item observed in the basic data of the earlier study was not found.

The results of the statistical analysis of the fertility data show a significant ( $< 5.0$  per cent level of probability), though small, difference in fertility in favor of the control bulls over the wheat germ oil-fed group. These results are not interpreted as indicating a depressing effect of wheat germ oil on the fertility of the bulls to which it was fed. However, no benefit to

TABLE 5  
*Fertility of the experimental bulls*

Wheat germ oil-fed bulls				Control bulls			
Bull No.	No. Services	No. conceptions	% conceptions	Bull No.	No. services	No. conceptions	% conceptions
1E	379	170	44.9	1C	303	126	41.6
2E	611	320	52.4	2C	1024	601	58.7
3E	345	133	38.6	3C	641	294	45.9
4E	450	274	60.9	4C	86	58	67.4
5E	298	177	59.4	5C	889	412	46.3
6E*	364	143	39.3	6C	576	309	53.6
7E	121	54	44.6	7C	382	220	57.6
8E*	219	73	33.3	8C*	61	24	39.3
9E	460	244	53.0	9C*	125	65	52.0
10E	434	235	54.1	10C	478	273	57.1
Total	3681	1823	49.5	Total	4565	2382	52.2

\* Dropped from experiment after varying periods of time for low fertility or refusal to use the artificial vagina.

fertility resulted from supplementing the ration composed of common, natural feeds with the wheat germ oil. The addition of wheat germ oil to the ration was ineffective in preventing a decrease in fertility in two bulls which finally had to be withdrawn from use in artificial insemination.

In spite of the fact that an effect of season on the quality of semen produced by the bulls was shown, the fertility of the same semen did not show correlated trends. The statistical analysis showed no significant difference in fertility between months.

#### SUMMARY

Two comparable groups of 10 bulls each were selected. Both were fed a practical ration made up of common, natural feeds which supplied plenty of vitamin E for normal reproduction of rats. To determine whether or not additional vitamin E in the form of solvent process wheat germ oil would benefit the reproductive performance of bulls used extensively for artificial

insemination, each of the bulls in one group received one ounce daily of the wheat germ oil during an experimental period of one year. Over 1,250 semen samples were collected from the 20 bulls, and over 8,200 cows were artificially inseminated during the experiment.

A comparison of the results from the two groups warrants the following statements:

The feeding of one ounce daily of solvent process wheat germ oil in addition to the normal ration did not:

1. Increase the volume of semen produced by the bulls;
2. Increase the spermatozoa concentration in the semen;
3. Improve the motility of the spermatozoa;
4. Shorten the time required for service;
5. Decrease the number of semen ejaculates which were discarded as being of too poor quality for use in artificial insemination;
6. Improve the fertility of the bulls to which it was fed;
7. Nor prevent two bulls from decreasing in fertility to such low levels as to force their withdrawal from use in artificial insemination.

A study of the seasonal effects showed a highly significant decrease in percentage of motile spermatozoa during the early spring months and a highly significant difference between months in spermatozoa count. The lowest average count was found in August, but there was no significant difference in fertility of the bulls from month to month.

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## RETENTION OF MOLD FRAGMENTS BY BUTTER, BUTTERMILK AND WASH WATER DURING MANUFACTURE OF BUTTER\*

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In earlier studies on factors affecting mold content of cream and butter (5) it became apparent that more information was needed on retention of mold fragments by butter during churning and subsequent operations. The question of mold retention is of considerable importance since the validity of any mold fragment (mold mycelia) count on butter depends upon the percentage and uniformity of mold transfer from cream to butter during churning. If the percentage of mold carried over to butter were low, or more important, if the percentage carried over were quite inconsistent from one churning to another, a count of mold fragments in the butter could not provide a reasonable index of the mold content of the cream. If such were the case, use of the Wildman mold mycelia method as an index of mold content and quality of raw cream would be entirely unjustified.

Results of two previous investigations on this problem do not agree. Wildman (6) reported results of an experiment in which four samples of cream were churned and mold mycelia determinations run on buttermilk and butter. Mold mycelia counts on the butters were 76, 40, 92 and 68 and on the respective buttermilks 4, 4, 4 and 24. Naturally the results could not be quantitative because the Wildman method does not attempt to measure total mold filament in a sample. However, in one churning in which their yield was measured, 2 parts of butter to 3 of buttermilk were obtained. The butter had a mold mycelia count of 76 per cent and the buttermilk 4 per cent. A reconstituted sample consisting of 2 parts of the melted butter and 3 parts of the buttermilk showed a mold mycelia count of 60 per cent. Wildman concluded that churning actually concentrated the mold in the butter.

Adams and Parfitt (1) concluded that mold mycelia retention by butter of mold mycelia in cream was in general between 20 and 30 per cent. *Oospora lactis* strains were retained from 9 to 42 per cent with an average retention of 23 per cent. From the standpoint of dead mold fragment studies on butter, these results must necessarily be discounted because they are based on agar plate counts and no consideration was given to weights or volumes of butter, buttermilk and wash water involved.

In view of its significance and the disagreement between studies thus far on this subject, it seemed advisable to investigate it further.

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## EXPERIMENTAL

Investigations were first carried out on laboratory churnings in quart mason jars. In order to more closely simulate the degree of agitation encountered in commercial churns, 1600-ml. quantities of sour cream containing varying amounts of mold were neutralized, pasteurized and then churned in gallon-size Dazey churns. The studies were then continued on commercial churnings. When this was done, a sample of the neutralized, pasteurized cream was collected as it went into the churn, and 1600 ml. of this sample also churned in a Dazey churn. Experimental lots of cream were churned at about 45° F. The temperature of these lots increased slightly during churning. The commercial lots were churned at about 48-50° F. About 25-30 minutes were required for the experimental churnings and 45 for the commercial. Some exceptions occurred and will be noted. A record of pounds or grams of cream, butter, buttermilk and wash water was kept for every churning. Because the amount of mold in the wash water was so small, only the first lot of wash water was analyzed for each of the commercial churnings. Only one washing was made in the case of laboratory churnings. In every churning an attempt was made to wash with a volume of water equivalent to that of the cream churned.

Estimation of total combined length of mold fragments in butter, buttermilk and wash water was made by the new quantitative method described in a previous paper (4). Some modifications in technique were necessary for buttermilk and wash water. Buttermilk was diluted at the rate of one gram per 19 ml. and wash water at the rate of 5 grams per 5 ml. hot gum solution. Cream was diluted in the same manner as butter. Total length of filament in all samples was calculated in terms of mm. per mgm., then total mm. of mold per gram or pound of sample calculated, and the total for the respective butter, buttermilk, wash water or cream in any one churning was determined. The percentage of the total visible mold filament contained in each portion of a churning was determined from the sum of that accounted for in butter, buttermilk and wash water. This procedure was considered preferable to using the cream count for the total because cream counts were less accurate than the others. Special methods of heating and agitating the cream samples are necessary to break up clumps of mold fragments.

It should be pointed out also that the counts included only visible mold fragments and not the tiny segments of *Oospora lactis* sometimes known as spores. In some trials attempts were made to include the tiny segments by using 500× magnification. They were significant in contributing to buttermilk and wash water, but not butter counts. However, the mold fragments that would contribute to an official mold mycelia count on butter were of primary interest and the studies therefore confined largely to fragments that might affect this value. Fragments 20 to 30 microns in length



were easily detected in butter and somewhat less readily in buttermilk. They were included in the counts. The tiniest fragments or so-called spores of *O. lactis* were about 10 microns in length.

Results in tables 1 and 2 indicate that in both laboratory and commercial churnings the mold fragments are predominantly retained by the butter. On a mgm. basis the total filament in butter usually ran several times greater than in buttermilk. Wash water generally contained much less mold than buttermilk. When weights of the different materials (butter, buttermilk and wash water) in a churning were taken into consideration, the butter in almost every case contained the majority of the mold that could be accounted for in all three materials. The total filament in butter, buttermilk and wash water usually approximated, roughly, the amount in the cream.

It was obvious that most of the longer mold filaments remained in the butter. There were many more long filaments in the butter than in the

TABLE 1  
*Total visible mold fragments retained by butter, buttermilk and wash water during laboratory churning*

Churning	Sample	Total visible mold	Grams of sample	Per cent of total visible mold
		<i>mm. per mgm.</i>		
1	Butter	14.98	585	68.52
	Buttermilk	4.15	930	30.22
	Wash water	0.10	1560	1.26
2	Butter	28.92	400	64.14
	Buttermilk	5.71	1115	35.33
	Wash water	0.60	1560	0.53
3	Butter	154.58	375	79.50
	Buttermilk	11.69	1165	18.68
	Wash water	0.87	1535	1.82

other materials. The shortest filaments of about 10–50 microns in length were most numerous in the buttermilk although many were also observed in the butter. In some churnings where the low fat content or other factors increased the churning time and agitation considerably, it was observed that the filaments were for the most part shorter and retention in the butter was lower. The percentage of total mold in buttermilk and wash water was greater than usual in such cases. No. C-2 in table 2 is an example of such a churning.

The mold content of the cream does not seem to have affected appreciably the percentage retained in the butter, buttermilk or wash water, respectively. In table 1 the third churning contained many times as much mold filament as the others and yet the percentage retained in the butter is not significantly greater than in the first two churnings. Similar results may be noted in table 2. Controlled studies on this one phase might be

desirable. The length of filaments and amount of agitation during churning probably determine more than the mold content of the cream how much mold will be distributed between butter and the buttermilk in a churning.

There is some indication that a high fat cream results in more mold being retained in the butter than when a low fat cream is churned. This

TABLE 2

*Total visible mold fragments retained by butter, buttermilk and wash water during commercial churning*

Churning	Sample	Total visible mold	Grams of sample	Per cent of total visible mold
		<i>mm. per mgm.</i>	<i>(× 454)</i>	
A-1	Butter	25.37	400	64.77
	Buttermilk	5.54	900	31.83
	Wash water	0.41	1300	3.40
B-1	Butter	13.25	635	92.42
	Buttermilk	.52	1265	7.23
	Wash water	.017	1900	0.35
C-1	Butter	15.24	945	81.82
	Buttermilk	1.56	1755	15.53
	Wash water	0.17	2700	2.65
B-2	Butter	15.33	822	72.91
	Buttermilk	3.46	1278	25.62
	Wash water	0.12	2100	1.47
C-2	Butter	10.74	504	48.95
	Buttermilk	3.46	1296	40.61
	Wash water	0.64	1800	10.44

may be related to churning time, amount of agitation required to bring about the reversal of phases and possibly other factors.

Judging from results in table 3 the Dazey churn provided about the same degree of agitation as the commercial churns since the total mold filament contents of butters obtained by the two methods were equivalent in every case.

TABLE 3

*Total visible mold fragments in butter from laboratory and commercial churnings on same lots of cream*

Churning	New quantitative method		Wildman method	
	Laboratory	Commercial	Laboratory	Commercial
	<i>mm. per mgm.</i>	<i>mm. per mgm.</i>	<i>per cent</i>	<i>per cent</i>
A	22.78	25.37	86	88
B-1	12.04	13.25	77	81
B-2	15.76	15.33	68	72
C-1	15.85	15.24	56	46
C-2	11.35	10.74	46	56

An interesting contrast is brought out by table 4. Percentage retention of bacteria differs greatly from that of the mold. The same slides as were

used for mold counts served also for bacterial counts. The oil immersion objective was used, however, for counting bacteria. The average number per field was multiplied by the microscopic factor to obtain the number per gram as for the microscopic count per ml. of milk (2). The percentages retained in the butter, buttermilk and wash water, respectively, were thus determined. The bacteria stained quite intensely and were easily recognized. Starter was added to the cream before churning lots C-1 and C-2 on which the bacterial study was made, and therefore sufficient bacteria were present to enable a fairly accurate study. That the bacterial counts were accurate is indicated by the fact that the total number accounted for in butter, wash water and buttermilk just about equaled the number in the cream before churning.

TABLE 4

*Bacteria retained by butter, buttermilk and wash water during commercial churning*

Churning	Sample	Direct micro- scopic count of bacteria	Grams of sample	Per cent of total number
		<i>no. per gm.</i>	<i>(<math>\times 454</math>)</i>	
C-1	Butter	25,980,000	955	1.15
	Buttermilk	1,065,180,000	1755	87.75
	Wash water	87,466,000	2700	11.09
C-2	Butter	8,660,000	504	0.75
	Buttermilk	415,680,000	1296	91.57
	Wash water	25,114,000	1800	7.68

The counts in every case indicated that bacterial cells tended to pass out into the buttermilk and also many more were removed by the wash water. An interesting fact in this connection was that the bacteria, which appeared to be a large-celled strain of *Streptococcus cremoris*, formed chains of cells which were in some cases longer than the filaments of mold retained by the butter. The chains of bacterial cells were about one-third to one-half the diameter (width) of the mold filaments.

#### DISCUSSION

The reason for the striking retention of mold filament by butter during churning is not entirely clear. Apparently the longer mold filaments are enmeshed or held by the fat phase when butter is formed during churning. These may be broken up to some extent during the ensuing working of the butter (3). It would seem that more of the long chains of bacterial cells might also be retained by the butter unless some difference in constitution, either physical or chemical, plays a part. Such explanations as effect of electric charge of bacteria and fat globules can only be speculative.

In evaluating the results, some consideration should be given to breaking up of filaments during churning and subsequent loss of the tiny fragments

in the buttermilk. This undoubtedly occurred to some degree and its extent could not be accurately determined by the counting methods used. Such a loss, if it could be determined, would actually lower the percentage figure retained for butter and increase that of the buttermilk. Studies with higher magnification indicated that when the shortest fragments were included, the retention figure for butter was lowered about 10–20 per cent and that of the buttermilk increased by this much. However, as mentioned earlier, these fragments would not contribute to the official mold mycelia count on butter and therefore were of less interest than the longer ones.

The results indicated that for studies of this nature the Dazey paddle churn provided conditions at least roughly approximating commercial churns. Since the total mold content of experimental and commercial butters from the same cream were about equal, the degree of agitation in the two types of churning must have been similar. This might not be true of churnings where quart mason jars are agitated to produce the butter.

For the most part the results substantiate the early report of Wildman regarding retention of mold by butter. It appears that most of the total mold filament is retained by the butter and that a high mold content cream is likely to produce a high mold content butter.

Nevertheless, such factors as fat content of cream, churning time and possibly others may be significant in affecting the carry-over of mold from cream to butter. The results indicate that in some churnings the variation in retention is enough to appreciably affect the mold mycelia count of butter. This is of significance in assessing the quality of butter on the basis of its mold mycelia count, particularly where rigid standards are enforced and butter consequently confiscated when it slightly exceeds the legal limit set by enforcement officials. Two lots of cream of equivalent mold content might not yield butters with the same mold mycelia count, if factors affecting retention enter into the picture. More data based on quantitative studies are needed on the significance of these factors.

#### SUMMARY

Total visible mold filament was determined on butter, buttermilk and wash water of both laboratory and commercial churnings.

Results indicated that butter usually retained more than 50 per cent of the total length of mold filament and that the wash water contained a very small percentage. The butter appeared to retain the long filaments during churning and most of the tiny fragments passed out into the buttermilk.

Studies on some commercial churnings indicated that butter retained a very small percentage of the bacteria of the original cream. Most of the bacteria were found in the buttermilk. This occurred in spite of the fact that many chains of the bacteria approximated the mold filaments in size.

The total mold content of laboratory churned butter approximated that of commercially churned from the same respective lots of cream.

The possible effects of other factors such as fat content of cream and degree of agitation during churning on retention of mold fragments by butter are briefly discussed.

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# UTILIZATION OF UREA AND GROWTH OF HEIFER CALVES WITH CORN MOLASSES OR CANE MOLASSES AS THE ONLY READILY AVAILABLE CARBOHYDRATE IN THE RATION\*

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In previously published studies on the utilization of urea by the ruminant, we presented data showing the need for a readily fermentable carbohydrate (4) in the ration, and for a low level of dietary protein (8), in order to have effective transformation of the urea nitrogen into protein. Corn starch was the carbohydrate studied. When urea was added to the basal ration of timothy hay only, hydrolysis of the urea to  $\text{NH}_3$  and disappearance of the  $\text{NH}_3$  from the rumen contents were very slow, and six hours after feeding there was no increase of the protein level of the rumen contents above that when the timothy hay alone was fed. When starch and urea were fed the protein level of the rumen contents rose rapidly and the ammonia nitrogen disappeared in six hours.

The question still remained whether a more soluble carbohydrate, such as that in molasses, would be as effective as starch in allowing utilization of the urea nitrogen. If it could so function it might be possible in sugar-producing countries to construct economically suitable rations for growing calves or milking cows from roughage plus urea and molasses, adequately fortified with bone meal, salt, and vitamin A if needed. The present experiment was set up with this purpose in mind. Corn molasses was used rather than cane molasses in order to provide a more rigorous test, since the corn molasses is practically free of nitrogen.

## EXPERIMENTAL

For this study we used a 1,000-pound Holstein heifer with a rumen fistula equipped with a removable rubber plug to facilitate sampling. The animal was fed the experimental ration twice daily, at 8 A.M. and 6 P.M. Samples for analysis were taken from the rumen at intervals of 1, 3, and 6 hours after the morning feeding. Sampling was done twice weekly, on Monday and Friday, for a period sufficiently long to give constant results for several days. A period of three weeks after a change in ration was always allowed for an adjustment period before studies on the rumen con-

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tents were undertaken. The samples were taken by mixing the top solid matter adjacent to the fistula with the more liquid ingesta underneath until a uniform consistency was obtained, and then withdrawing a sample of about a kilogram. Four hundred grams of the sample were used for a dry matter determination, and 20-gram samples were used for triplicate determinations of urea, ammonia, and total nitrogen. The urea was determined by the urease method and the ammonia by magnesium oxide distillation. Urea nitrogen, ammonia nitrogen, and protein were calculated as per cent of the total dry matter present. The protein was calculated as (total nitrogen minus  $(\text{NH}_3\text{-N plus urea-N}) \times 6.25$ ).

The daily experimental rations in the order of feeding were as follows:

*Period 1*—Timothy hay alone (12 pounds). The hay for this and all subsequent periods was cut to lengths of  $\frac{1}{2}$  inch or less by running through a hammer mill.

*Period 2*—Timothy hay (10 pounds) + corn molasses (4 pounds).

*Period 3*—Timothy hay (10 pounds) + corn molasses (4 pounds) + urea (200 grams).

*Period 4*—Timothy hay (10 pounds).

*Period 5*—Timothy hay (10 pounds) + commercial corn starch (2 pounds) + corn molasses (2 pounds).

*Period 6*—Timothy hay (10 pounds) + starch (2 pounds) + corn molasses (2 pounds) + urea (200 grams).

*Period 7*—Timothy hay (10 pounds) + starch (2 pounds) + corn molasses (2 pounds) + casein (0.4 pound).

*Period 8*—Same as period 7 + urea (200 grams).

*Period 9*—Timothy hay (10 pounds) + corn molasses (4 pounds) + urea (200 grams).

## RESULTS

The results are summarized in table 1. The figures given are the average analyses of samplings on three or four separate days. The  $\text{NH}_3$  + urea-N and total protein values are given as per cent of the dry weight for one, three and six hours after feeding.

The chief point to be observed is that the combination of timothy hay, molasses, and urea (Period 3) gave a protein level of the rumen contents of 9.5 to 10.0 per cent, somewhat lower than the level of 10.75–11.0 per cent protein obtained on feeding a similar ration in which starch had been substituted for one-half of the molasses (Period 6). In addition, the ammonia nitrogen disappeared more slowly on the hay, molasses, and urea ration than it did when starch was added, remaining at a level of 0.09 per cent at six hours, as contrasted with 0.024 per cent.

Results with the other rations, which acted as controls for the above two rations, were as expected, both the protein and  $\text{NH}_3\text{-N}$  remaining at low levels.



The addition of casein to the hay, starch and molasses (Period 7) raised the protein level of the ingesta to 9 per cent, as would be expected. The further addition of urea (Period 8) then caused the protein level to rise to 11 per cent of the total dry matter. The protein did not rise above this level, even though plenty of  $\text{NH}_3$  was available, as shown by the fairly high  $\text{NH}_3\text{-N}$  at six hours. As previously shown (8), the effectiveness of urea utilization depends upon the level of preformed protein in the diet; the more protein above a certain level fed, the less urea will be utilized in raising the protein of the ingesta to the maximum.

When it was evident from the results just discussed that molasses was at least fairly effective in aiding urea utilization, it was decided to determine whether growing calves could meet their protein requirements largely from urea, with molasses as the carbohydrate source. Three heifer Holstein calves

TABLE 1  
*Influence of rations on urea utilization in rumen of fistula heifer*  
Per cent on dry basis

Period	Ration	$\text{NH}_3\text{-N} + \text{Urea-N}$			Total protein		
		1 hr.	3 hrs.	6 hrs.	1 hr.	3 hrs.	6 hrs.
1	Timothy hay .....	0.036	0.041	0.025	7.56	7.83	7.72
2	Timothy hay + molasses ..	0.009	0.010	0.008	6.58	6.17	6.52
3	Timothy hay + molasses + urea .....	0.139	0.153	0.090	9.79	9.98	9.28
4	Timothy hay .....	0.033	0.024	0.020	6.15	6.62	7.62
5	Timothy hay + starch + molasses .....	0.018	0.013	0.010	7.47	7.04	7.85
6	Timothy hay + starch + molasses + urea .....	0.158	0.150	0.024	11.06	10.77	10.90
7	Timothy hay + starch + molasses + casein .....	0.030	0.025	0.014	8.95	9.02	9.11
8	Timothy hay + starch + molasses + casein + urea ..	0.184	0.220	0.094	10.08	11.10	11.00
9	Timothy hay + molasses + urea .....	0.146	0.141	0.076	9.35	9.69	9.37

weighing about 200 pounds each were placed on a ration of the following composition:

Timothy hay .....	49
Cane molasses .....	60-70
Bone meal .....	2.0
Urea .....	2.5
Iodized salt .....	1.0
Shark liver oil capsules .....	

The total protein equivalent in the ration (nitrogen  $\times$  6.25) was 11.6 per cent. The timothy hay (6 per cent protein) supplied approximately 3 pounds and the cane molasses (1.6 per cent protein) from 0.96 to 1.02 pounds of protein per 100 pounds of ration. Seven to eight pounds of protein equivalent were supplied by the urea. Consequently the urea fur-

nished from 60 to 65 per cent of the total nitrogen in the ration. The cane molasses contained 71.1 per cent of solids and in these growth studies was used instead of corn molasses. The shark liver oil was furnished at a level supplying 1500 International Units per day per calf. The ration was thoroughly mixed except for the shark liver oil which was fed daily in capsules. The ration was a sticky, uninviting mixture, but in spite of this there was no failure of ready consumption. It was fed at an increased daily rate as determined by complete consumption. The calves were weighed weekly. Some milk was also fed for the first three weeks until the calves became accustomed to the ration. The molasses was increased from 60 to 70 parts after seven weeks when it became apparent that a maximum rate of growth was not being attained, possibly because the caloric intake was insufficient for their needs.

During a period of 19 weeks on the above ration the calves gained a total of 102, 104, and 82 pounds, respectively, or 0.77, 0.78, and 0.62 pounds per day, or about half the normal rate of growth. The weights of the calves after this period were 337, 290, and 267 pounds, respectively. See table 2 for summary of all of the growth data.

TABLE 2  
*Showing rates of daily gain on the different rations*

Ration	Gains in wt. (lbs. daily)		
	No. 1	No. 2	No. 3
Timothy hay + cane molasses + urea + bone meal + iodized salt + vitamin A .....	0.77	0.78	0.62
Timothy hay + cane molasses + urea + 0.3 pounds casein + bone meal + iodized salt + vitamin A .....	1.7	1.5	1.6
Timothy hay + cane molasses + urea + 0.3 pounds starch + bone meal + iodized salt + vitamin A .....	1.3	1.4	1.5
Timothy hay + corn molasses + urea + starch + bone meal + iodized salt + vitamin A .....	1.4	1.5	2.0

It seemed evident that normal growth could not be obtained with such a ration as timothy hay, supplemented with molasses and urea. Since the intake of urea was large it was probable that the proteins of the microorganisms in the rumen were furnishing the larger share of the amino acids necessary for growth. We have other data involving urea feeding which show that a total protein equivalent in the ration of 9.49 per cent (2) was sufficient for growth in calves at a rate of 1.0-1.2 pounds per day. *Such rations always contained a certain amount of natural protein derived from yellow corn.* Consequently we were inclined to believe that the proteins produced on the ration used in these later experiments were more likely deficient in quality than in quantity of total protein. With this point in view, we added 0.3 pound of casein per day (commercial crude) to the ration of each calf in order to determine whether the slow growth was due to a

protein deficiency. This addition raised the total protein equivalent in the ration to 13.5 per cent on the basis of a daily consumption of 14 pounds of the basal ration.

Over a period of seven weeks No. 1 gained 83 pounds (daily average 1.7 pounds), No. 2 gained 72 pounds (daily average 1.5 pounds), and No. 3 gained 78 pounds (daily average 1.6 pounds). These results indicate that the molasses, urea, and timothy hay mixture was either not able through the proteins of microorganisms to provide protein of best quality for optimum growth or to provide sufficient protein. While the total protein equivalent of the ration without casein was 11.6 per cent, comparing well with that of successful rations made of urea, starch, *corn meal* and timothy hay, it must be kept in mind that in an open system such as prevails in the ruminant's tract, some of the molasses sugar may have moved out of the rumen faster than was the case with starch. This would account for the somewhat lower protein in the rumen content and explain why an addition of 0.3 pound of casein daily increased markedly the rate of growth.

After seven weeks on the ration containing casein the latter was withdrawn and in its place 0.3 pound of corn starch was substituted daily. Because of the shortage of cane molasses it was only possible to make growth observations over a span of 4 weeks. In this period No. 1 gained 36 pounds, equivalent to 1.3 pounds per day. No. 2 gained 40 pounds, equivalent to 1.4 pounds per day. No. 3 gained 41 pounds, equivalent to 1.5 pounds per day. The average daily gain of the three calves in this period was 1.4 pounds as compared with 1.6 pounds daily during the period of casein feeding, but it was a much better rate than secured on the molasses ration alone and compared favorably with the gains on the molasses-casein ration. Evidently the addition of the small quantity of starch had allowed a more efficient protein manufacture to proceed—probably due to the continuous hydrolysis of the starch and a more continuous source of soluble sugar.

To test further the hypothesis that with carbohydrate and urea an effective protein mixture for normal growth could be secured, the three calves were changed to the following ration:

42.25	pounds timothy hay
32.75	pounds starch
20	pounds corn molasses
2	pounds bone meal
2.5	pounds urea
1	pound iodized salt

Vitamin A was furnished as shark liver oil in gelatin capsules. No other protein source was available except that of the timothy hay. Corn molasses was used to improve the palatability and also to introduce a sugar containing no nitrogen. On this ration No. 1 gained 81 pounds in 8 weeks equivalent to 1.4 pounds per day. No. 2 gained 82 pounds in 8 weeks equivalent

to 1.5 pounds per day. No. 3 gained 114 pounds in 8 weeks equivalent to 2.0 pounds per day.

These are considered normal rates of gain for this species. It is evident that a ration carrying, in addition to the roughage, only molasses with a small amount of starch or the reverse can adequately function with urea as the main source of nitrogen. Further, when rumen conditions are optimal for the growth of the microorganisms the amount and quality of the proteins produced can cause a maximum rate of growth of heifer calves.

#### DISCUSSION

From the results of the first phase of the feeding experiments it seemed possible that not enough protein was formed in the rumen and what was formed may have been of poor quality. As stated above, it has been shown (2) that calves will grow almost normally on rations where the only nitrogen sources were yellow corn, starch, timothy hay, and urea, and at a protein equivalent level of 9.49 per cent. This seemed to indicate that the protein formed from the urea by the microorganisms in the rumen was of fair quality but that the corn proteins may have acted as effective supplements. In the case of lambs, Harris and Mitchell (1) reported that the addition of urea to a ration unable to support appreciable growth converted the ration into one capable of promoting a nearly normal rate of growth. In all of our work on growth or milk production with calves or cows the greatest effectiveness of urea was obtained only in the presence of certain grain proteins and starch.

The experiments outlined in this paper would indicate that proteins of high quality and sufficient quantity can be formed in the rumen by the microorganisms there, provided some slowly hydrolyzable carbohydrate is provided, such as starch. A roughage plus urea and a soluble sugar alone will be less effective. No doubt our early success in attaining normal growth of calves with a ration containing corn meal and starch was mainly due to the provision of a slowly hydrolyzable carbohydrate such as the starch in the corn meal or starch itself.

These results also raise the question as to the validity of the idea that the biological importance of protein is of less significance in the case of the ruminant (7) than with other animals. At the moment the idea prevails that the ingested proteins in the case of the ruminant are largely converted to microorganism proteins and these then serve as the primary amino acid source for the animal. This idea bans the older conception that liquids and finely ground feeds largely pass directly through the esophageal groove into the lower digestive tract.

The experiments outlined in this paper contribute nothing definite toward an answer to that problem. However in 1915 (3) Hart and Humphrey published data showing that for maintenance and milk production in

cows the proteins of milk showed a definitely greater efficiency than those of corn or wheat. Positive nitrogen balances were maintained with milk proteins for the production of as high as 35 pounds of milk per day but negative balances resulted when the proteins at the same level were derived from the corn or wheat grain. Such results indicate that all ingested protein is not reworked to the protein of the microorganism but that at least some of that in finely divided condition may go directly through the rumen to the lower digestive regions.

The results of the fistula experiments indicate that only partial utilization of urea by ruminants occurs when molasses is the chief source of readily fermentable carbohydrate. Protein is formed in the rumen, but the final level of protein reached is not as high as when a less soluble carbohydrate is in the ration. This does not mean that the combination of urea and molasses is ineffective, but that it is not as efficient as a combination of urea and starch. The calf growth experiment indicates the same fact. These results agree with those of Pearson and Smith (6), who found that upon the incubation of rumen ingesta *in vitro* with urea and various carbohydrates, starch was most effective in causing synthesis of protein, as indicated by the decrease in NPN in the medium. Galactose and maltose were also good, sucrose was fair, while dextrin, glucose, glycerol and lactic acid were relatively poor.

In another paper of the same series (5), they conclude that *in vivo* experiments of the type reported here cannot be expected to yield any certain evidence of protein synthesis from urea until truly representative samples of total ingesta can be obtained and analyzed, and until more is known of the effect of urea on the passage of the various dietary constituents through the rumen. We agree that obtaining representative samples of the entire rumen is very difficult, and that little is known of the rate of passage of various constituents out of the rumen. However, we believe that reliable results can and have been obtained with our technique in spite of these difficulties. Instead of trying to obtain samples representative of the entire rumen content, we took samples from the same spot every time, made as similar as possible by controlling the moisture content. By this method samples can be taken from day to day that vary only slightly in percentage composition; triplicate determinations on the same sample check very closely.

#### SUMMARY

1. Further studies on urea utilization in a rumen fistula heifer are reported.

2. With timothy hay as the sole ingredient of the basal ration utilization of urea was low. Corn molasses provided a suitable substrate for the development of an active flora, and urea was fairly well utilized. The

protein of the rumen contents (dry basis) rose from a basal level of 7.7 per cent to 9.28 per cent.

3. With timothy hay, starch, corn molasses and urea as the ration the protein level in the rumen contents rose from 7.7 per cent to 10.9 per cent. Apparently somewhat better utilization of urea was made on a starch-containing ration than on one containing mainly a more soluble sugar.

4. In growth experiments with young heifer calves a ration made of timothy hay, *cane* molasses and urea fortified with common salt, bone meal, and vitamin A gave a subnormal rate of growth (0.6 to 0.8 pound daily).

5. When this ration was supplemented with 0.3 pound of crude casein daily normal growth was attained. Substitution of an equal weight of corn starch for the casein likewise resulted in normal growth.

6. For maximum growth of calves a ration made of a roughage, molasses and urea must be supplemented with some additional source of a more insoluble but fermentable carbohydrate or insoluble protein which can be drawn from the cereal grains or concentrates, such as the oil meals. In our limited experience this supplemental material need not be more than 3-5 per cent of the total ration.

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## RUMEN SYNTHESIS OF THE VITAMIN B COMPLEX AS INFLUENCED BY RATION COMPOSITION\*

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Since Bechdel *et al.* (2) reported that cattle, and possibly all other ruminants, possess the ability to synthesize the vitamin B (complex) in the rumen, workers have been interested in the problem from the following points of view. First, what effect do the constituents of the ration have upon the synthetic powers of the organisms involved and the quantity of vitamin produced; second, what types of organisms are responsible for these syntheses. Wegner *et al.* (21) reported that cows are able to synthesize thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, and biotin; and further, the addition of thiamine to the ration apparently increases the synthesis of the above members of the B-complex except nicotinic acid. They (22) also found that increased nitrogen as added casein in the ration did not increase synthesis; in fact it decreased it in the case of riboflavin. McElroy and Goss (10, 11, 12, 13, 14, 15), working on the same problem, reported that sheep and cows were able to synthesize riboflavin, pyridoxine, thiamine and pantothenic acid on vitamin-low rations. Jukes and McElroy (15) have also shown that biotin was synthesized in the rumen. Hunt *et al.* (4, 5) reported that there was a direct correlation between riboflavin synthesis and the carbohydrate in the ration, and that *ground* yellow corn was better than whole corn as a substrate in the synthesis of riboflavin. They were unable to detect any thiamine synthesis in their experiments as judged by the comparative amounts in the ration and the rumen at various time intervals after feeding. On an exclusive alfalfa hay ration they found less riboflavin in the dried ingesta of the rumen than in the hay; suggesting either that the riboflavin was rapidly absorbed by the animal or in part destroyed. Kiek and associates (7) reported that the rumen contents of steers fed alfalfa hay exclusively were alkaline in reaction, whereas if grain and a protein supplement were fed with alfalfa hay the rumen contents were acid in reaction. These results would in part explain the low values reported above for riboflavin since it is destroyed in an alkaline medium. The normal pH of the rumen will vary with the ration, but is usually between 6.8-7.3. McElroy and Goss (12) reported that adjusting the pH of the rumen samples to

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4.5–5.0 with concentrated HCl before drying enhanced the riboflavin values. Wegner *et al.* (22) reported that adjusting the pH to 4.5–5.0 did not increase the riboflavin values as was reported by McElroy and Goss.

In this paper there is reported the effect of nitrogen added to the ration as urea and the extent of the vitamin B-complex synthesis in the rumen of the fistulated cow and calf. The aim was to determine if there was any correlation between added nitrogen and carbohydrate on the vitamin synthesis in the rumen.

#### EXPERIMENTAL

The animals were fed the following rations for a period of one month.

##### Ration

1. Timothy hay (12 lbs.)
2. Timothy hay (10 lbs.)    Corn molasses (4 lbs.)
3. Same as No. 2 plus 200 grams of urea per day.
4. Timothy hay (10 lbs.)
5. Timothy hay (10 lbs.)    Corn molasses (2 lbs.)    Starch (2 lbs.)
6. Same as No. 5 plus 200 grams of urea per day.
7. Timothy hay (10 lbs.)    Corn molasses (2 lbs.)    Starch (2 lbs.)    Casein (acid washed) (0.2 lbs.)
8. Same as No. 7 plus 200 grams of urea per day.

At the end of each period a sample of the rumen content was removed for vitamin assay. The samples were very constant in composition on the basis of moisture content. The sample and an amount of 95 per cent ethyl alcohol, equal in volume to the weight of the sample, were placed in the cold room, 7° C., for one week to stop bacterial action and fermentation. The contents were then placed in enamel trays and dried at 37–40° C. The dried samples were ground in the Wiley mill and assayed for biotin (16), nicotinic acid (8, 18), pantothenic acid (20), riboflavin (17, 19), thiamine (3), folic acid<sup>1</sup> (9), and pyridoxine (1). All of the constituents of the ration were assayed for the above vitamins. (See tables 1, 2 and 3.)

#### RESULTS

The addition of nitrogen, as urea, resulted in increased synthesis of nicotinic acid, biotin, riboflavin and pantothenic acid in the rumen, but significantly only when molasses or a readily fermentable carbohydrate was supplied with the ration.

The synthesis of pyridoxine could not always be correlated with the variation in ration composition. However, in some instances a correlation was obtained, as for example, with rations 3 and 6. In these instances the addition of urea to the ration containing a fermentable carbohydrate definitely increased the synthesis of pyridoxine.

<sup>1</sup> The folic acid values are calculated as follows:

$$\frac{\% \text{ activity}}{4} = \gamma/\text{gram}$$



There was no direct correlation between increased urea intake and vitamin synthesis in respect to "folie acid."

TABLE 1

*Cow—vitamin content per gram of dry rumen material*

Ration	Thia- mine	Ribo- flavin	Nico- tinic acid	Panto- thenic acid	Biotin	Folie acid	Pyri- doxine
	$\mu\text{g./gram}$	$\mu\text{g./gram}$	$\mu\text{g./gram}$	$\mu\text{g./gram}$	$m\mu\text{g./gram}$	$\mu\text{g./gram}$	$\mu\text{g./gram}$
1	0.17	6.7	31.0	3.4	207	0.42	2.6
2	0.24	7.6	39.6	3.9	253	0.33	2.6
3	0.36	13.6	64.7	12.0	277	0.42	4.2
4	0.18	7.7	35.2	5.8	207	0.52	2.6
5	0.18	5.3	30.9	5.7	212	0.31	2.5
6	0.08	12.7	65.6	17.9	289	0.57	4.3
7	0.12	11.5	56.6	18.2	250	0.34	2.8
8	0.35	12.0	59.0	16.3	309	0.49	2.6

TABLE 2

*Calf—vitamin content per gram of dry rumen material*

Ration	Thia- mine	Ribo- flavin	Nico- tinic acid	Panto- thenic acid	Biotin	Folie acid	Pyri- doxine
	$\mu\text{g./gram}$	$\mu\text{g./gram}$	$\mu\text{g./gram}$	$\mu\text{g./gram}$	$m\mu\text{g./gram}$	$\mu\text{g./gram}$	$\mu\text{g./gram}$
1	0.39	7.4	24.3	5.3	210	0.41	3.2
2	0.14	5.2	31.0	5.2	224	0.33	2.0
3	0.46	10.3	49.6	10.8	299	0.37	3.9
4	0.69	9.7	40.4	9.5	188	0.64	3.3
5	0.45	8.6	40.4	5.8	200	0.24	2.7
6	1.00	15.7	57.2	13.3	307	0.33	3.6
7	0.59	11.6	56.8	17.8	240	0.55	2.8
8	0.65	12.8	70.8	18.0	294	0.49	2.8

TABLE 3

*Vitamin content of ration ingredients per gram of dry material*

Material	Thia- mine	Ribo- flavin	Nico- tinic acid	Panto- thenic acid	Biotin	Folie acid	Pyri- doxine
	$\mu\text{g./gram}$	$\mu\text{g./gram}$	$\mu\text{g./gram}$	$\mu\text{g./gram}$	$m\mu\text{g./gram}$	$\mu\text{g./gram}$	$\mu\text{g./gram}$
Timothy hay .....	1.8	5.5	23.5	8.6	62.2	.....	3.9
Corn molasses .....	< 0.1	< 0.05	< 0.05	< 0.05	< 10.0	< 0.1	< 0.1
Corn starch .....	< 0.1	< 0.05	< 0.05	< 0.05	< 15.0	< 0.1	< 0.1
Urea .....	< 0.1	< 0.05	< 0.05	< 0.05	< 12.0	< 0.1	< 0.1
Casein—acid washed	< 0.1	< 0.05	< 0.05	< 0.05	< 20.0	< 0.05	< 0.05

In the case of thiamine and with a non-synthetic type of ration we encountered the same effect as observed by Hunt *et al.* (4, 5). The value

obtained indicate that there was little, if any, synthesis of this essential vitamin. It was reported by Kennersley *et al.* (6) that thiamine is oxidized in alcoholic solution if allowed to stand for several months. To test this point four samples of rumen contents were dried directly, omitting the alcoholic storage, and the same values as shown above were obtained, indicating no destruction by storage in alcohol.

Timothy hay is the only constituent of the ration that contained any appreciable amount of the above vitamins (table 3).

#### SUMMARY

The addition of urea as a source of nitrogen definitely increased the synthesis of riboflavin, nicotinic acid, biotin and pantothenic acid in the bovine rumen when a readily available carbohydrate was present. Pyridoxine and "folic acid" could not be too closely correlated with ration composition. In the absence of a readily fermentable carbohydrate and probably a low population of microorganisms the synthesis of the members of the B complex is not at a maximum.

The data indicate that thiamine may not be synthesized in the rumen. However, it seems more than probable that it is synthesized, but absorbed or destroyed at a rate greater than its synthesis.

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## CAROTENE LOSSES IN FRESHLY CUT PLANT TISSUES\*

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The carotene in hays and silages is the most important source of vitamin A for dairy cows. The amount of carotene in hays is largely dependent upon the curing process. Hays may contain relatively small amounts of carotene even though they were made from fresh, green plant materials with high initial carotene contents. In studying this loss of carotene during the hay-curing process Hauge (8) obtained evidence for the presence of a carotene-destroying enzyme in alfalfa. That there may be differences in enzyme activity in different plants is indicated by the work of Bolin (3) who found that when fresh plant material was stored at five degrees F. for ten months, the loss of carotene in alfalfa was 62.7 per cent while there was little or no loss in brome grass, meadow fescue, orchard grass, and Kentucky blue grass. During the summer of 1941 the authors found that bluegrass, even though dry and brown in color due to lack of rainfall, contained a surprisingly large amount of carotene. This suggested the possibility that bluegrass might be low in the carotene-destroying enzyme. In studies with grasses and berseem, Seshan and Shen (12) concluded that neither mold or bacterial action affected carotene losses and apparently doubted that enzymes have any great effect upon carotene losses in these plants. With these facts as a basis, it seemed desirable to study the losses of carotene in various plant materials under conditions which were favorable to enzyme activity and under other conditions which inhibited enzyme actions.

### EXPERIMENTAL

The relative enzyme activity of various plant materials was determined by the loss of carotene during an incubation period at a favorable temperature. The difference in loss of carotene between two samples, the enzymes having been inactivated by heat in one and not in the other, may be attributed directly to the effect of the enzymes. Some samples were incubated in an atmosphere of nitrogen. Any decreases in carotene losses of autoclaved samples incubated in an atmosphere of nitrogen are probably due to inhibition of uncatalyzed oxidation. Any decreases in carotene losses of samples not autoclaved but incubated in an atmosphere of nitrogen should be due to inhibition of uncatalyzed oxidation and also to inhibition of catalyzed oxidation if the enzyme is aerobic.

The plant materials, which had been secured fresh from the field, were immediately chopped into quarter to half-inch lengths and mixed. In the

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first series of experiments weighed samples were placed into a series of stoppered test tubes and treated as follows:

1. Fresh material, no treatment.
2. Incubated 24 hours at 37° C. in a hot air oven.
3. Autoclaved five minutes in steam at 115° C., followed by incubation for 24 hours at 37° C.
4. Incubated 24 hours at 37° C. in an atmosphere of nitrogen.
5. Autoclaved five minutes at 115° C., followed by incubation for 24 hours at 37° C. in an atmosphere of nitrogen.
6. Autoclaved five minutes at 115° C.
7. Dried in vacuum oven at 100° C. for 24 hours.

The carotene content of these samples was determined by a modification of Moore's method (9). After the carotene had been transferred from the alcohol-petroleum ether solution into petroleum ether, the solution was saponified by shaking in a separatory funnel for about one minute with 25 ml. of 25 per cent potassium hydroxide in methyl alcohol. This was followed by washing with water until free of alkali and alcohol, drying with anhydrous sodium sulfate, and passing the petroleum ether carotene solution through a dicalcium phosphate column as described by Moore.

The results of these experiments are shown in table 1. It was found that the loss of carotene upon incubation of the fresh material was greatest in alfalfa and red clover and lowest in oat, bluegrass, and brome grass. However, in the samples which were autoclaved before incubation a high percentage of the carotene was retained in all cases. The differences in losses between the two treatments indicated that the chief losses during treatment 2 were due to the enzyme activity in the plants, while the variation in losses indicates considerable variation in enzyme activity of the different plant materials studied.

Inactivation of enzymes by autoclaving or vacuum drying did not greatly affect the carotene content. This shows that the carotene in plant materials is fairly stable to these heat treatments.

Samples incubated in an atmosphere of nitrogen retained a high percentage of their carotene. This indicates that the enzyme is probably aerobic and may be the same as the one described by Haas and Bohn (7) and studied by several other workers (1, 2, 5, 6, 10, 13, 18). Further evidence of this relationship was found when aqueous extracts of alfalfa, red clover and bluegrass were tested for carotene-destroying enzymes by the method of Reiser and Fraps (10). It was observed that the destruction of carotene in these solutions was similar to that observed in the plants. This indicates that the carotene-destroying systems in the different plants are similar.

Autoclaved samples lost some carotene upon incubation which indicates that non-enzymic oxidation occurred. This may explain why some of the

TABLE 1  
*Micrograms of carotene per gram of plant material (dry basis) after the indicated treatments to show variations in the content of carotene destroying enzymes*

Date 1943	Material	*Treatment of sample						Remarks
		1 (*F)	2 (I)	3 (A, I)	4 (I, N)	5 (A, I, N)	6 (A)	7 (VD)
9-27	Alfalfa-leaves and stems	454.3	88.2	321.6	210.5	401.4	487.6	411.4
6-21	Red clover leaves	491.3	137.9	405.5	464.5	525.3	508.3	First growth
7-1	Seresia lespedeza-leaves	281.3	171.5	239.6	250.1	268.5	273.2	Height—2'
7-15	Swiss chard	465.4	216.6	415.3	457.9	451.9	385.5	Height—12"
7-5	Corn-leaves	563.6	248.2	435.8	494.1	447.0	465.9	Height—4'
6-15	Oat	387.8	317.1	333.9	360.6	362.6	373.2	Height—8"
7-19	Bluegrass	528.9	405.6	462.0	486.6	498.3	540.9	Clippings
6-24	Bromegrass	736.0	558.1	624.0	652.9	668.4	621.6	Height—6"

\* F—fresh.  
 I—incubated 24 hours at 37° C.  
 A—autoclaved 5 minutes at 115° C.  
 N—air replaced by N.  
 VD—vacuum dried 24 hours at 100° C.

samples incubated under nitrogen were higher in carotene than those incubated in air following autoclaving.

In order to eliminate any effect that the process of autoclaving might have upon carotene losses, a series of experiments was conducted in which samples were both incubated and autoclaved. One sample of the material was autoclaved and then incubated, and the other was incubated and then autoclaved. Thus each sample received the same treatment some time in the procedure and consequently any differences in the carotene contents may be ascribed solely to the effect of the enzyme. The results of these experiments are shown in table 2. It again becomes evident that there is considerable variation in the enzyme activity of plants.

TABLE 2

*Micrograms of carotene per gram of plant material (dry basis) after incubation with and without enzyme inactivation*

Date 1943	Material	Treatment		Per cent carotene destroyed	Remarks
		*AI	IA		
7-22	Corn-leaves .....	519.8	264.9	49.0	Height—3'
7-22	Ladino clover-leaves .....	104.0	29.8	71.4	Second growth
8-6	1st year sweet clover .....	86.0	12.9	85.0	Height—1'
8-6	Korean lespedeza-stem and leaves .....	263.5	212.0	19.5	Height—4"
8-9	Soybean-leaves .....	432.8	368.6	14.8	Bloom stage
8-9	Bluegrass .....	548.8	439.0	20.0	Clippings
8-11	Timothy .....	245.9	150.9	38.7	First year growth
9-7	Corn-leaves .....	381.0	252.2	33.8	Height—8" Early dent stage
9-7	Korean lespedeza-stem and leaves .....	219.0	201.9	7.8	Height—6"
9-3	Soybean-leaves .....	315.0	188.2	40.1	Pod stage

\* AI—Autoclaved 5 minutes at 115° C. followed by incubating 24 hours at 37° C.

IA—Incubated and then autoclaved.

These experiments verify the earlier observations of Hauge (8), who concluded that enzyme action was responsible for a considerable portion of the large initial loss of carotene in alfalfa which follows the cutting of the plant.

The data presented indicate that certain plant materials, because of their low enzyme activity, should lend themselves more readily than others in grass mixtures for the production of hays and silages of high carotene content. Undoubtedly there is considerable enzymatic destruction of carotene during the wilting of some plant materials before ensiling and therefore reduction of the interval of time between cutting and ensiling should result in silage of higher carotene content. Russel *et al.* (11) found that in the field-curing of hay the carotene losses were greatest immediately following cutting and the rate of losses was closely correlated with conditions favorable to enzyme action. Dehydration of the plant material is one of the factors



slowing the enzyme action. Camburn *et al.* (4) found greater percentage loss of carotene in sun-cured than artificially dried hays during storage. This indicates that the enzymes are active in dry stored materials. If this is true, the advantage of inactivating the enzyme or having material with low enzyme content is apparent.

Further investigations should be made to study factors which affect the enzymatic activities of plant materials to obtain information that would be helpful in conserving the carotene in hay, silage and certain dehydration products.

#### SUMMARY

Studies have been made to determine the losses of carotene in freshly cut plant materials under conditions which were favorable to enzyme activity and under other conditions which inhibited enzyme action.

Evidence is presented which indicates that the destruction of carotene, due to enzymatic activity, is greater in alfalfa, red clover, and sweet clover than in the oat plant, Kentucky bluegrass and brome grass. Other plants such as corn, soybeans, and lespedeza seem to have an intermediate enzyme activity. The enzyme appears to be aerobic in character.

Although the carotene losses in wilted plant materials are related to enzyme activity, some non-enzymic destruction also occurs.

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# JOURNAL OF DAIRY SCIENCE

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## PAPERS PRESENTED AT THE ANNUAL MEETING

### SYMPOSIA PAPERS

#### I. RELATION OF BODY SIZE TO MILK YIELD

- A. The Energy-Size Basis of Measuring Milk Yield. W. L. GAINES,  
*University of Illinois, Urbana, Ill.*

This paper presents some material bearing on the validity of using  $FCM_s/W_1$  as a criterion of dairy development or lactational drive in dairy cows and goats.  $FCM_s$  is milk-energy yield in pounds four per cent milk per day for the first eight months (243 days) of lactation and  $W_1$  is live weight of the cow or doe within 31 days after parturition for the same lactation— $FCM_s/W_1$  to be measured for each lactation of each cow or doe.

It is shown that  $FCM_s$  is practically unaffected by age independent of  $W_1$ . On the other hand,  $FCM_s$  is profoundly affected by  $W_1$  independent of age. Ignoring age  $FCM_s$  tends to be proportional to  $W_1$ . Direct test shows that  $FCM_s/W_1$  is independent of age, hence age corrections are eliminated. Direct test shows also that disregarding age  $FCM_s/W_1$  is independent of  $W_1$ . These statements apply to dairy cows and does separately, not both together.

In the data presented there appears to be a ceiling for  $FCM_s/W_1$  at about 55 in cows and 110 in goats; also, average  $FCM_s/W_1$  is about twice as high in goats as in cows. That is, with respect to  $FCM_s/W_1$  or metabolic intensity: cows are *like cows*; goats are *like goats*; cows and goats are *different*.

A breeding philosophy: A, breed for size, criterion  $W_1$  (large or small as desired); B, breed for milk composition, criterion fat percentage (high or low as desired); C, breed for lactational drive, criterion  $FCM_s/W_1$  (high as feasible). A, B and C are mutually independent in inheritance. The main thought is to bring dairy development into sharp focus through the quantitative measure  $FCM_s/W_1$ .

A tentative standard of excellence in the progeny test of a bull: 1000  $FCM_s/W_1 = 40$  as an average of his daughters; as a plurality of daughter lactations becomes available, variance between daughters is not substantially greater than that within daughters (or intraclass correlation = 0). The first indicates a high level of dairy development, the second indicates homogeneity.

To estimate gross efficiency ( $100 \times \text{milk calories} / \text{digestible feed calories}$ ) the following formulas are indicated:

Cows,  $62.5 (1000 \text{ FCM}_s / W_1) / (80/3 + 1000 \text{ FCM}_s / W_1)$

Does,  $62.5 (1000 \text{ FCM}_s / W_1) / (160/3 + 1000 \text{ FCM}_s / W_1)$

Two serious objections to gross efficiency are: it is subject to a large error of estimate; it largely obliterates differences between individuals, particularly at high levels of  $\text{FCM}_s / W_1$ .

## B. The Significance of Body Size in the Economy of Milk Production.\*

SAMUEL BRODY, *Department of Dairy Husbandry, University of Missouri, Columbia, Mo.*

Body size affects the economy of milk production. This fact is generally known, but it is confused by associated factors which we should like to clarify.

1. *"Dairy merit": definition.*<sup>1</sup> One reason for the confusion surrounding the effect of body size on the economy of milk production is lack of clear-cut definitions. The designation "dairy merit" is related in most minds to the economy of milk production, but the relation is not clear; it means different things to different men. Let us, therefore, define it sharply by saying that "dairy merit" represents the biologic efficiency of milk production as measured by the percentage of consumed TDN energy which is converted into milk (FCM) energy. The ceiling of "dairy merit" is 50 per cent; not over  $\frac{1}{2}$  or 50 per cent of the consumed TDN may be recovered as milk energy. (The other 50 per cent of the consumed TDN is expended for maintenance and related processes.) The dairy merit of "good" dairy animals is about 25 per cent, that is, they convert  $\frac{1}{4}$  or 25 per cent of the TDN energy into milk energy; the dairy merit of "superior" animals is about 33 per cent, that is they convert  $\frac{1}{3}$  or 33 per cent of the consumed TDN energy into milk energy.

2. *Body size and dairy merit.* Dairy merit appears to be<sup>1</sup> independent of body size *as such*. Rats, dairy goats, and dairy cattle of all sizes may convert up to about 50 per cent of the ingested TDN energy into milk energy.

3. *Body size and monetary profit of milk production.* If 1600-lb. and 800-lb. cows are in the same dairy merit class, both, for example, converting 30 per cent of the consumed TDN energy into milk energy, and if other conditions are equal, the profit on the milk produced by the 1600-lb. cows will be much greater than on the 800-lb. cows. This is because the labor cost of milking, feeding, cleaning, housing, bookkeeping, etc., is nearly half as much for one 1600-lb. cow as for two equally efficient 800-lb. cows.

This may be illustrated by a more specific numerical example. Suppose that it is desired to produce 1000 lb. FCM daily at 30 per cent efficiency.

\* Contribution from the Dept. of Dairy Husbandry, Mo. Agr. Exp. Station Journal Series No. 948.

<sup>1</sup> Mo. Agr. Exp. Sta. Res. Bull. 366, 1943.

By definition of "efficiency" (ratio of milk energy produced to TDN energy consumed), the same amount of TDN, namely,<sup>1</sup> 625 lb., will be consumed to produce this 1000 lb. FCM regardless of the size of the animals. But the number of animals required to produce 1000 lb. FCM per day at 30 per cent efficiency will differ with their size; by our method of computation<sup>1</sup> (FCM yield varies with  $W^{0.73}$ ) it will require fifty-three 700-lb. cows but only twenty-six 1700-lb. cows to produce the 1000 lb. FCM at 30 per cent efficiency. By Gaines' method of computation (FCM yield varies directly with  $W^{1.0}$ ) the difference would be still greater, it would require twice as many 800-lb. cows as 1600-lb. cows to produce 1000 lb. FCM. Since it requires about twice as many stanchions and twice as much labor to handle 53 than 26 cows, the overhead costs (housing, milking, labor, and so on) will be roughly one-half and the profit correspondingly greater in producing the milk with the 1700-lb. than with the 700-lb. cows of *equal dairy merit*. In other words, a given number of large animals constitute a larger business with greater profits than small animals of the *same dairy merit*.

It was shown<sup>1</sup> by a typical numerical example, involving payment for labor, management, and housing, and when *all other conditions are equal*, that the profit on 1400-lb. cows (30 of which are required to produce 1000 lb. FCM at 30 per cent efficiency) is about 50 per cent greater than on 900-lb. cows (42 of which are required to produce FCM at 30 per cent efficiency). Needless to say that "all other conditions" are never equal. There are differences in market demand and in price; in topography and climate; in ability to graze on rolling country and ability to withstand heat and drought; in clumsiness of larger animals; in greater efficiency of smaller animals as result, not of body size *as such*, but because of the more intensive selection that has been practiced on the smaller animals, and so on, which may nullify, in part, the above generalization concerning the greater profitableness of large animals under a given set of conditions. This, then, is in the nature of a general rule, and like other general rules has many exceptions. The importance of these exceptions to this general rule will be reduced in proportion to the increase in equitable standardization of milk quality, standardization in feeding, management, and housing conditions, and standardization in selection and breeding on the basis of dairy merit (rather than on the basis of absolute milk yield, which is ambiguous because of its dependence on body size as well as on dairy merit).

**C. Body Size and Lactation Rate.** MAX KLEIBER, *Division of Animal Husbandry, College of Agriculture, University of California, Davis, Calif.*

The influence of age on lactation rate can be determined when the effect of body size is derived independently as on the basis of the theory that lactation capacity is proportional to the metabolic body size ( $\frac{3}{4}$  power of body weight) of the cows.

The effect of body size on lactation rate cannot be calculated from records within one herd because; (1) the variability of lactation rate, aside from influences of size, ranges from  $\pm 10$  to  $\pm 20$  per cent of the mean rate even in well-bred herds kept under uniform conditions, and (2) size differences in those herds are as a rule relatively small. The unreliability of results from a recent attempt to calculate size effects on lactation rates within a herd is demonstrated, and a table is given showing the number of cows necessary to distinguish significantly between production rate per unit weight and production rate per unit of metabolic body size.

A table is supplied in which metabolic body size can be read directly when body weight in pounds is given.

Lactation rate per unit of metabolic body size is a sound basis for the calculation of age effects on lactation rate.

The average daily milk production during a 10-month period, expressed as milk energy, and divided by the mean metabolic body size, is suggested as an important result in summaries of production records. When the cows have been kept under *quasi* ideal conditions such a result may be known as relative lactation capacity, expressing quantitatively the inherent ability of cows for milk production and thus useful as a major criterion for breeding dairy cattle.

## II. POSTWAR PROBLEMS OF DAIRYING

### A. Post-War Problems of Dairying. ERNEST L. ANTHONY, *Dean of Agriculture, Michigan State College, East Lansing, Mich.*

It is already certain that our agricultural colleges will bear much of the brunt of the post-war thinking and planning as it will effect the policies and changes in the Agriculture of the United States at the close of the war. In nearly every agricultural college in the United States may be found, today, faculty committees busy at this task. There is some tendency in the public mind to belittle post-war planning efforts on the basis that the first job is to win the war. No one will disagree that the first objective of all of us is to win and stop this needless war as soon as possible, but to so win the war and lose the fullest benefits of the first years of peace by unpreparedness is foolish indeed.

*Post-War Planning Should Become Post-War Preparedness.* It is certain that American Agriculture will, as it did following World War No. I, feel the full effect of production to the peace time demands of a war-weary world.

The dairy industry stands today in the front rank of all American farm products in its war role. This industry, thus, has been called upon to expand to the utmost. It stands today at the peak of its production and demands.

To visualize the future which confronts the dairy industry, I feel several things must be given careful consideration. I have listed them as they appear important to me. Undoubtedly many others should be discussed.

1. *What will be the future effect of the widespread recognition of dairy products as essential in good nutrition?*

Today, while prices are good, the industry should use a large percentage of its price gains to more fully educate all the public to the continuing place which dairy products have in a rational adequate diet program for the nation.

2. *What about the post-war competition of other foods?*

*The dairy industry is in for keen competition.* This competition will be from within and from without.

3. *What about our consumer relation in the post-war period?*

The dairy industry, in its rapid growth, has been so busy just producing more and more that it has paid but little attention to its consumers.

The future security of its present importance depends upon a loyal satisfied consuming public. *It does not have it now.*

4. *Will the dairy industry face a lower price structure?*

The greatest inflation in the industry at present is in cattle prices. The future of pure bred cattle is at stake in the present activity.

5. *What about Research in the Dairy Industry?*

No other industry has benefited more from the results of fundamental and practical research than has the dairy industry. On the other hand, as an industry it has done the least in its own behalf.

## **B. Is Dairy Expansion to Continue?** EARL WEAVER, *Michigan State College, East Lansing, Mich.*

Certain data relating to dairy production, prices and income in the United States for the 34-year period 1910 to 1943 inclusive are presented. These data attest that dairy production in this country has shown a quite consistent increase. The average annual total milk production in the United States for the 34-year period has been 90,644,000,000 pounds. In 1910 the production was only 72 per cent of this average; it increased to the peak of 132 per cent in 1942.

But certain facts here are commonly overlooked. In the 34-year period the incline in production has been interrupted on only three occasions. In a war period, which is accompanied by general agricultural prosperity, relative interest in dairy production subsides. The curve flattened in the years of World War I. Production also dropped from 1942 to 1943 in spite of enormous pressure for increased milk production during the latter year. Even greater pressure is being exerted in 1944. Approximately a million dollars a day in incentive payments to dairy producers have been spent since last October, but expected production this year will not change greatly from that of 1943 and will be less than 1942.

The third interruption in the inclining curve was in 1934 and was due to the drouth that year with the extreme shortage of feed and the consequent slaughter of cows.

Data pertaining to the price of milk are also shown. For the 34-year period the average price has been \$2.23 per hundred pounds for milk. This ranged from the highest price for 1919 of 152 per cent of the average down to the low point for 1932 of 61 per cent of the average. In 1943 it was back up to 139 per cent of average and will be higher in 1944 because of the incentive payments.

Several observations can be drawn from these data on milk prices but the most significant observation is that these prices have no direct correlation with total milk production. Most persons anticipate a decline in the general price level in the postwar period. Obviously milk prices will drop too but the data here indicate it is erroneous to conclude therefrom that milk production will also drop. On the contrary there appears a tendency for an acceleration in milk production under the conditions that may be anticipated in the postwar period.

Other data confirming this latter observation are shown. As stated, in years of agricultural prosperity relative interest in the dairy enterprise tends to subside. Conversely, in depression years relative interest in dairying increases.

A curve is presented to show the annual gross farm income in the United States from 1910 to 1943, the average being \$10,850,247,000. A companion curve is also carried to show the per cent of the gross farm income that was due to the sale or home use of dairy products. On the average 16.1 per cent of the gross has been due to these dairy products. During World War I gross farm income rose to 157 per cent of the average, but dairy products contributed only about 12 per cent of the gross. In the two years following that war gross farm income dropped from 157 per cent of the average down to 92 per cent. In the same period there was a rise from 12 per cent up to 16.8 per cent in the portion of the gross that was due to dairy products.

The depression year of 1932 marks the low point in gross farm income. It was only 55 per cent of the average. In that year, the per cent of the gross that was due to dairy products rose to 22.4, the highest percentage in history.

There is full recognition of the fact that no one can predict with certainty the conditions that will confront the dairy industry in the postwar period. However, I venture my interpretation of the data as follows and I perceive if these predictions materialize the resulting condition will bring more serious problems than the dairy industry has ever before encountered.

1. The general price level as well as dairy prices will decline.
2. Even with such declines it should not be surmised that milk production in this country will subside.



3. On the contrary, as all farm enterprises become less remunerative, there will be relatively more interest in the dairy enterprise to utilize most effectively the farm-grown feeds and the abundant farm labor likely to be available.

4. With this continued liberal milk production in the face of likely curtailed consumer purchases, profit margins will be reduced and in many cases will disappear.

5. One solution to the likely problems lies in the dairy producer's ability to breed, feed and manage his dairy herd with a degree of efficiency not heretofore achieved generally.

6. Another opportunity to help solve the problems lies in sincere appreciation by producers and processors of the necessity of choicest quality in dairy products. This and the consequent elimination of inferior products from consumer channels are indispensable if the industry is to survive the impact of the postwar period.

7. No group of persons involved in the dairy industry will face more strenuous demands for service nor be able to render greater immediate service than the extension workers—those identified with colleges, the breed fieldmen, plant fieldmen and all who are devoted to dairy educational work.

**D. Postwar Demand for U. S. Livestock Abroad.** O. E. REED, *Chief, Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A., Washington, D. C.*

It is difficult to make a reliable appraisal of the postwar demand for purebred livestock in Europe and South America; first, because the postwar agricultural and economic conditions cannot be determined at this time, and secondly, because we have very little information about the present status of the livestock situation abroad, particularly in those countries that have been overrun by the enemy.

We do know, however, that livestock numbers have been reduced substantially in northwestern Europe, where normally there is heavy dependence on imported feed grains and oil cakes. Inability to import these feeds means that the greatest reductions have occurred in hog and poultry numbers. Sheep numbers by contrast have been well maintained.

Piecing together all available information, it appears that for the European continent as a whole the decline as of 1943 was about 10 to 15 per cent below 1939 in the case of cattle, and approximately 30 to 35 per cent in the case of hogs. Sheep numbers may even have increased.

As a result of these developments, the productive capacity of the European livestock industry has been accordingly reduced. Total meat production is estimated at about 70 per cent of prewar production; eggs at about 60 per cent; whole milk for fresh consumption at about two-thirds of prewar; cheese at about two-thirds; and butter at approximately 80 per cent.

The knowledge that European countries have suffered considerable losses in livestock numbers naturally has created interest among livestock men and others in the United States. Consequently, there has been much discussion about the role this country may expect to play in restocking and developing the livestock industries in Europe. Many statements have been made expressing an optimistic view of the possible demand for our livestock. In my opinion, such statements have been based on wishful thinking rather than on a practicable consideration of the probabilities.

In any event, I doubt that there will be any great demand on United States farmers and livestock breeders in the near future. Even if hostilities should cease in Europe in 1944, it would be a year or more before the governments of the depleted countries could make arrangements to import any considerable number of livestock.

Also, there seems to be a widespread notion among some of our breeders of registered dairy cattle that such stock will be in great demand when rehabilitation programs are under way. In talking to many of the representatives of the occupied countries who have expressed an interest in obtaining dairy cattle when the proper time comes, I find that they are interested primarily in obtaining cows for utility purposes and they are not interested in registered breeding stock, except possibly a few outstanding proved sires. It is well to remember in this connection that most of the European countries that plan to import breeding stock will want the breeds they are now using. This is true of all kinds of livestock and consequently not many countries would be interested in the breeds we have to offer.

Apparently the first thing that will be given consideration by those in charge of rehabilitating the war-damaged countries will be the problem of obtaining food supplies for the starving populations as quickly as possible and in the most feasible manner.

No doubt the demand for food in all war-damaged countries will emphasize the need for livestock products, particularly dairy products. Consequently, emphasis will be placed upon the rebuilding of the livestock industries in these countries as soon as practicable. However, I do not believe there will be any sudden demand for large numbers of breeding stock from this country. Most of those with whom I have discussed the probable situation seem to agree that the rehabilitation of herd and flocks in the European countries after the war will be primarily a matter of encouraging the natural increase in numbers as soon as feed is made available, and that relief by livestock shipments from overseas will probably not be in great volume.

In regard to Latin America, the possible demand for our purebred dairy cattle is more favorable. But even this market would not call for a large number of breeding animals, at least not for some time. Demand for breeding stock of the dairy breeds is increasing in Latin American countries,

particularly in the tropical countries and in those on the Pacific side of South America. This demand probably will continue after the war as long as the purchasing power of those countries remains relatively good. But cattle buyers who come to us from these countries will need advice and help, so that they can obtain the right kind of cattle for their conditions at a reasonable price. Such assistance will be an important factor in developing this foreign market.

In many areas of Latin America, particularly in the tropical lowlands, our dairy breeds have not proved entirely satisfactory. In most instances the imported animals have had to face such obstacles as heavy infestation with cattle tick and other parasites, less satisfactory feeding and management than that to which they had been accustomed, as well as high environmental temperatures. Frequently, such conditions have been responsible for failures that were wrongly attributed to a breed's inability to withstand tropical conditions.

More research work will be needed to determine the effect of continued high temperature on the productive and reproductive performance of improved breeds of dairy cattle. The dairyman in these countries needs help in the way of education and research to enable him to create a better feeding and management environment in which to maintain the cattle he imports or the descendants of such cattle. This really ought to precede the importation of large numbers of cattle. In other words, it will first be necessary to assist dairymen in informing themselves as to latest and most modern methods of taking care of good cattle. The possibility of developing a suitable tropical dairy type by grading up the native stock with our purebred breeds should not be overlooked.

Education and research organizations and associations, such as the American Dairy Science Association, could do much to foster education and research in the dairy field in Latin America. Within the last two years the Bureau of Dairy Industry, in cooperation with the Department's Office of Foreign Agricultural Relations, has sent three men to a number of South and Central American countries to survey dairy conditions. Dr. Hodgson, who will discuss the dairy industries in Latin American countries on this program, was accompanied on one trip by Dr. Hunziker and on the second trip by your own president, Dr. Dahlberg.

These surveys have made us aware of the rapidly developing interest in dairying in Latin America, and also of the fact that there is a potential market for U. S. dairy equipment and supplies of all kinds, including some dairy breeding stock. Before this market will materialize, however, more attention will have to be given to educational and research programs in those countries in order to help the farmers and livestock men there acquaint themselves with modern ideas and practices relating to the dairy industry.

F. Nutrition Education as a Safeguard Against Postwar Surpluses. E.  
M. HARMON, *Director of Public Relations, National Dairy Council,  
Chicago, Ill.*

Aside from production shifts there are two factors which may make for dairy surpluses in the postwar period. The first of these is changing food habits on the part of consumers. The second is the return to our markets of products now going to foreign nations for lend-lease and other purposes.

In connection with the first, it is certain that food habits are changing. In an extensive survey by the *New York Times* recently, 75.8 per cent indicated changes in food habits due to rationing. Of these, 55.2 per cent indicated that those changes were substantial.

When asked what the changes were, 84.8 per cent said that the most important change was a shift to oleomargarine. This far exceeded any other food change.

The effect of this change in food habits may be gained from the realization that there are now only about 12 pounds of butter available per person per year. This compares with the about 17 pounds in the prewar period. That 5-pound drop represents considerably over 600 million pounds of butter which in terms of milk equivalent means more than 12 billion pounds of milk or more than 10 per cent of the nation's milk supply.

Nor is this the only food habit change that may plague the dairy industry when supplies are normal. Cheese consumption is cut from about 6½ pounds in the prewar period to an estimated 4.2 pounds available for 1944. Cured cheddar cheese is almost unknown, and if this condition prevails long enough the appetite for that type of cheese may be largely lost. Candy manufacturers are finding substitutes for dairy products. Bakers who were fast learning the value of milk solids have been forced to abandon them.

This is not the first experience of this kind that the dairy industry has encountered. The fat shortages in the first World War led to a reduction in per capita butter consumption from 18 pounds per year to 14 pounds per year. Five years were required to get back the normal butter consumption, and that was not nearly as drastic a shift in food habits as is the present one.

Nor is the dairy industry alone in this type of experience. Meat rationing in the form of meatless days in the last World War caused changes in food habits that have plagued the meat industry ever since. Wheatless days caused a permanent shift in food habits. According to the Bureau of Agricultural Economics we consume 18 per cent less wheat per capita than at the beginning of the first World War. The coffee industry, so far, has found itself unable to restore the consumption of the prerationing period. These observations lead to the question of: What shadows do the present changes in food habits cast upon the dairy horizons of tomorrow?

As an answer, some wishful thinking in the dairy industry forecasted vastly expanded European markets for many years to come. No one can

presume to have a positive answer on that point. Even could we know just what the dairy herd of Europe is today, the route or routes the invasion takes, the time required and the intensity of the fighting will all have a great bearing on what that herd is when the war ends.

The limited information available seems to indicate to date that the dairy herd of Europe is fairly well intact. Dr. Karl Brandt, head of the Research Foundation of Leland Stanford University, estimates the cow population is on the increase in many sections of Europe. Dr. Brandt estimates that while hog and poultry population have been severely cut, the productive dairy herd of Europe is probably not nearly as drastically reduced as supposed by many. Due to his close connections with agricultural economists in the continent of Europe and particularly in Sweden and Switzerland, Dr. Brandt is credited with being one of the best-informed men on European agriculture today.

The explanation is that Europeans have killed the grain-consuming animals. Since cows are not necessarily competitors with humans for grains but can be maintained on a subsistence ration of roughages they have been spared till the last. If these observations are correct, then it seems likely that Europe will come back to normal dairy production with reasonable rapidity once trade relations are open and their normal supply of 100 million tons of grain a year becomes available. Such would speed the day when the dairy industry of America must look to the home market as practically the sole outlet for its products.

Coming to the consumer, to whom we must look as the ultimate buyer for all of our dairy food products, even the most casual observer will note a receptive mind for nutrition information. Numerous factors have contributed to this situation—military draftee rejections, nutrition education in factories and war plants to overcome fatigue periods, accidents and absenteeism and the national nutrition program which is patterned largely upon the one which the dairy industry founded in 1918.

Every branch of the food industry is aware of this situation. Smart merchandisers know that food habits are changing and all are busy trying to either profit by those changes or to avoid any long time losses from them.

In this connection the educational and professional groups are confronted with difficult problems. Teachers have children in their school rooms from homes where both parents are working and where there is less chance to protect the child's nutritional habits than before. In factories and war plants individual nutrition specialists are charged with the responsibility of building and guiding nutritional habits which will make for maximum efficiency of workers. Doctors, dentists, dietitians, home economists, nurses and public health officials are confronted with the same problems.

All appreciate the merits of the slogan: "Make friends before you need them." These groups, charged with the responsibility of the education

and health of the nation, need friends and need assistance, and hundreds of thousands of them are finding at least some of that help in Dairy Council materials and the aid of the nutrition specialists of the Dairy Council units. A survey among 2600 teachers recently publicized in "Printer's Ink" emphasizes this point. Among this group 93.5 per cent wanted other teaching materials in addition to textbooks. Only half of them, however, found other such materials suitable. Such comments as "Too much sales promotion"; "Not geared to existing school programs"; "Not technically correct"; "Too much use of trade names" were prevalent. All of this points very definitely to two conclusions. First, practically all teachers want additional teaching helps, but, for the most part, industry has not been smart enough to provide them with the type of helps they need.

On the question of the type of subjects where they especially needed assistance, 76 $\frac{3}{4}$  per cent of them asked for food and nutrition material. This was far ahead of any other classification.

The National Dairy Council program is geared into just this kind of an outline. A committee of nationally known educators meets at least four times a year with the nutrition group of the National Dairy Council and a committee of local unit directors to advise with them as to just the type of educational materials most acceptable to schools and professional groups. This Advisory Committee includes an outstanding leader in the primary education field, another in the intermediate, and a third in the adult education field.

The same story will be told differently and with a different type of material for people of different age and education levels. For example, the story of calcium in milk would be told quite differently to the youngster in the primary grades than to the one in the intermediate grades and certainly far differently than to the dentist. This results in a course of study which lasts throughout the school period and on into the adult and professional groups. In this respect the dairy industry educational program is unique. Furthermore, all such materials are pre-tested under actual conditions.

The prestige of the Dairy Council has been gained due to the comprehensive yet unbiased nature of the nutrition education program of the dairy industry. As such it has become effective with nearly every educational, professional, civic, consumer and governmental group in any way interested in public health and welfare.

There are plenty of evidences of the value of nutrition education work throughout the nation. The recent New York *Times* survey mentioned earlier in this paper indicated that next to rationing, nutrition education is of the greatest importance in determining food habits. When we turn to the national picture the fighters' ration today indicates definitely that there have been some drastic changes in thinking during the past 25 years. In the first World War, 11 ounces of milk a day were required to make all of the

dairy products included in the garrison ration. In this World War 37 ounces of milk per day are required to make the dairy products in the garrison ration of one soldier.

Coming out to the local areas we find an even more impressive picture. In 1941 the Bureau of Agricultural Economics reported that 4 per cent more milk was sold in cities and villages than in 1940. A survey made by the National Dairy Council and including areas containing about 68 per cent of the entire population of the United States in cities of over 100,000 showed 6.2 per cent increase in cities having Dairy Council programs and a 1.5 per cent increase where there was no Dairy Council.

In the following year, 1942, compared with 1941 the Bureau of Agricultural Economics indicated an increase of 8 per cent in the amount of milk sold in cities and villages. Using the same procedure it was found that sales increased 10.9 per cent in cities maintaining active Dairy Council units and only 5.3 per cent in those not so doing. With the inauguration of the FDO orders sales restrictions were imposed in different areas during the latter part of 1943 so that this trend was somewhat checked.

This constant trend offers convincing proof of the importance of nutrition education work in the postwar plans of the dairy industry. Such a program, of course, should be backed up by additional research on the nutritional values of dairy products in experiment stations and laboratories. It will gain materially from the added impetus from advertising and point of sale efforts by the American Dairy Association as well as brand promotion by commercial organizations within the industry.

Steady, consistent, long-time gains and permanent stability of demand requires as a fundamental support a sound, comprehensive nutrition education which reaches the home from every angle—the teacher, the school, the dentist, the doctor, the public health official, the civic clubs and the women's organizations. Such a program should not be thought of as a cure-all, but it is a potential factor toward the relief of the surplus problems of the postwar era.

#### G. Postwar Problems in Country Collection and City Delivery of Milk.

LELAND SPENCER, *Cornell University, Ithaca, N. Y.*

The collection of milk from the farms and the delivery of milk to consumers in the city are at opposite ends of the chain of marketing operations. However, they have one thing in common. The main essential for low unit costs in each of these operations is large loads.

*Country hauling.* The average cost of hauling milk from the farms to dairy plants in New York State is about 16 cents per hundredweight, or 1/3 cent a quart. Most of the hauling now is done by commercial haulers. Only about one-fifth of the dairymen in New York State still haul their own milk to market.

Unit costs go up rapidly when loads fall below a reasonable level which depends upon the hauling distance and the size of truck being used. Hauling distances have increased due to the consolidation of country plants. On the other hand, larger loads are being hauled on the truck routes because fewer dairymen are attempting to haul their own milk. Consequently the rates charged for hauling held about steady until the last two or three years, during which time there has been an advance of 10 to 15 per cent.

Further economies in country milk hauling are to be sought mainly by reducing the number of haulers. The most practicable way of doing this appears to be through supervision of hauling arrangements by the producers' associations or the plants that receive the milk. Some cooperatives have been quite successful in obtaining rate reductions by contracting with the haulers, assuring them of good-sized loads and protecting them from the demands of individual producers for special service. This type of supervision is much to be preferred to the regulation of services and rates by public service commissions. It is practically impossible for public regulatory agencies to make proper allowances for the many local differences that have a bearing on milk hauling services and rates.

*City delivery.* The cost of delivering milk from the processing plant to the doorstep varies from about 4 cents to 7 cents a quart. The cost is higher in large cities than in smaller places and also tends to be higher in the North than in the South.

The principal element in the cost of milk delivery is the compensation of labor. Weekly earnings of driver-salesmen have gone up sharply in recent years. In New York City the average weekly earnings were nearly three times as much in 1943 as in the years immediately preceding World War I. Meanwhile the work week has been reduced from 7 days to 6, vacations with pay have been granted, and social security privileges have been provided.

Similar gains in the earnings of other groups of workers have been offset by increased output per man, but this was not true in milk delivery prior to the advent of every-other-day service. In fact, for many years prior to 1942, there was a gradual decline in the size of loads carried on retail milk routes in the large cities, such as New York and Chicago. The change from daily delivery to every-other-day service, which was made in 1942 and 1943, is by far the most important step ever taken to increase the size of loads and reduce the unit costs of retail milk delivery. Except in those markets where the earnings of driver-salesmen consist largely of commissions, the saving of manpower, together with the saving in truck expense, through every-other-day delivery has been sufficient to offset wartime increases in other costs. In some markets the distributors' spread has been reduced, while the drivers are earning more with fewer hours of work.

The most important question facing the fluid milk industry today is "How can the gains in efficiency of delivery through every-other-day service



be retained after the war?" Presumably the federal regulations under which every-other-day delivery was initiated and maintained during the war will not be continued in peacetime. When these regulations are discontinued, it is very probable that one or more distributors in practically every market will again offer daily service as a means of attracting additional customers. In order to cope with this type of competition, distributors who wish to maintain the E.O.D. system must offer suitable price concessions for the more limited service, even though temporary losses may result.

In the markets where the driver-salesmen are strongly unionized, much will depend upon the attitude of the union leaders. It seems clear that continuance of every-other-day delivery will be to the drivers' advantage. Daily service might seem to call for the employment of more drivers. However, if the E.O.D. system were abandoned, it would be impossible to maintain the present level of weekly earnings without a sharp increase in costs. The effect of this would be to accelerate the shift from retail delivery to store distribution, and from fluid milk to canned milk and dried milk, thereby causing a permanent reduction in the number of drivers employed.

In those states where milk control agencies have the authority to fix retail prices, lower minimum prices should be allowed where deliveries are made on alternate days.

### III. DEHYDRATED MILK AND MILK PRODUCTS

#### A. Present Developments of the Dried Milk and Milk Products Industries in the U. S. A. P. H. TRACY, *Department of Dairy Husbandry, University of Illinois, Urbana, Ill.*

The urgent need for dried food products for use by our Army and Navy and for shipment abroad has greatly stimulated our interests in the perfection of methods of manufacturing and packaging dried milk and related products. The production of nonfat dry milk solids has increased from 16,463,000 pounds in 1916 to 449,291,000 pounds in 1938. The demand for this product for use in the food industries will likely increase in the postwar period.

The future of dried butterfat in this country is uncertain. There is some question as to whether or not producers here will be able to compete with those of New Zealand and Australia in a postwar period devoid of trade barriers.

Much remains to be learned about the proper method to manufacture powdered ice cream mix but the indications are that the product will be one of considerable importance in the postwar period.

The production of malted milk has remained practically unchanged during the past 25 years. The consumption of this product increases in years of prosperity and decreases when business conditions are unfavorable.

The manufacture of dried whey has nearly trebled in the last five years. The less whey placed in the sewer or used for stock food and the higher the proportions used for human food or medicinal purposes, the more economical will cheese-making operations become.

The merits of dried buttermilk as a food for poultry and hogs has been recognized for some time. Sweet cream buttermilk has been found satisfactory for human food. From the standpoint of efficient utilization of milk solids, the establishment of whole milk creameries of such size that they can profitably manufacture dried defatted milk or sweet cream buttermilk seems a logical procedure.

The estimated production of powdered whole milk for 1943 was 124,300,-000 pounds or nearly 60 times that of 1916 and over 4 times the production of 1940. A problem for research workers is to perfect methods of manufacture and distribution that will result in a powdered whole milk, and possibly a powdered cream, that will have good keeping quality, that can be easily reconstituted, that will have a normal taste and feel in the mouth, that will have a normal appearance in the glass, and that can be sold at a price that will be attractive to the consumer.

The future of the dried milk industry will depend largely upon research. Both industrial and institutional research will be necessary to learn what should be known about the proper methods of manufacture, possible new uses of the dried milk and milk products, and a more complete knowledge of the nutritional properties of these products.

**B. Revision of Specification on Milk Powders.** LT. ROBERT REMALEY,  
*Subsistence Research and Development Laboratory, U. S. Army  
Quartermaster Corps.*

During the past sixty days, the Quartermaster Corps has published a new specification for dry whole milk and nonfat dry milk solids—No. 166A, dated 8 April. This specification supersedes all other specifications insofar as Army purchases are concerned.

Because of the more rigid storage requirements of the Army and because of the expansion of production of milk powders, the new specification controls more closely the four factors which appear to be of the greatest importance in the production of a palatable milk powder; namely, moisture, iron and copper contamination, raw milk supply and the oxygen content of the gases surrounding the packaged product.

Controlled laboratory studies as well as milk powders returned from the field have indicated that when the requirements of the new specification have been complied with, that whole milk powder will remain palatable for as long as 18 months even under adverse conditions of storage. Laboratory experiments have also indicated that as the temperature of storage increases the necessity for proper control of the four factors mentioned increases.

Variances in result of testing laboratories on identical samples are beyond the normally expected experimental error. Increased numbers of samples and inexperience of the analyst probably account for some of this variation. As a result, two programs are being initiated by the Quartermaster Corps Subsistence Research and Development Laboratory. One program, under the supervision of the American Dry Milk Institute, is an intensive study involving 576 determinations on identical samples in 8 laboratories on fat, moisture, titratable acidity and bacterial count in order to establish experimental errors. The other program is more extensive and involves all analytical procedures, which are required by the new specification. Over twenty-five laboratories including Government, Army, Universities and Industry are collaborating.

C. Dehydrated Milk and Milk Products, Use of Antioxidants. S. T.

COULTER, *Dairy Division, University of Minnesota, St. Paul, Minn.*

The prevention of tallowiness is a major factor in the storage of dry whole milk and other high fat dehydrated milk products. Although not today added commercially to dry milk, antioxidants are used in some fatty foods.

The following statements with respect to the effectiveness of various antioxidants in dry whole milk are based primarily on published experimental work. (1) Oat flour added in amounts equivalent to about 2 per cent of the weight of the milk solids delays the onset of tallowiness. (2) Hydroquinone in amounts equivalent to 0.01 per cent of the weight of the milk solids is definitely antioxygenic, but its use has been objected to by Pure Food and Drug Officials. (3) Wheat germ oil concentrates added at the rate of 0.1 to 0.2 per cent of the weight of the fat is comparable in effectiveness to hydroquinone. The antioxygenic activity of wheat germ oil is enhanced by the addition of citric acid. (4) Ascorbic acid at the rate of about 0.1 per cent of the weight of the milk solids retards oxidation. This is doubtless an example of the synergism that has been shown to exist between the tocopherols and many acids. (5) Gum guaiac and nordihydroguaiaretic acid are effective antioxidants but may cause the development of a fruity flavor. (6) Other substances have been shown to have some antioxygenic effect in dry whole milk. Much experimental work on antioxidants is now in progress. More effective antioxidants may be found.

Milk itself contains appreciable quantities of natural antioxidants, notably tocopherols and ascorbic acid. Reducing substances which are definitely antioxygenic may be produced in milk by heat treatment. The preservation of the natural antioxidants together with the use of processing treatments to secure the optimum antioxygenic effect should be the first recourse of the industry.

**D. Army Packaging and Packing of Dried Milk Products.** MAJOR JAMES D'A. CLARK, *Subsistence Research and Development Laboratory, U. S. Army Quartermaster Corps.*

Present Army requirements for dried milk products are discussed, including skim-milk powder, whole-milk powder, ice cream powder, and dehydrated cheese, together with a presentation of Army requirements for labeling, packing, and marking of shipping cases. The reasons for the various requirements are briefly reviewed with comments on the possibility of improving the keeping quality of whole dried milk which is, for many reasons, a product of more than usual importance.

**E. The Future of the Dehydrated Milk and Milk Products Industry.** J. T. WALSH, *American Dry Milk Institute, Chicago, Ill.*

War conditions have placed a sudden emphasis on the importance, adaptability, and nutritional value of foods—especially non-fat dry milk solids, dry whole milk, dry ice cream mix—for use by the Armed Forces and by Lend-Lease.

The dry milk industry, while yet a young, robust, and growing industry, has been established on a sound basis for approximately the past 20 years. It cannot be regarded as only a "war baby," which, of course, cannot be said of many types of dehydrated food products. The dry milk producers have invested much money in research development, and market expansion for their product. Their foresight and the soundness of their program, both in the past and at present, is readily reflected in Government set-aside requirements for Armed Forces and Allied uses. The industry has gained consumer acceptance of its products, all of relatively high nutritional value.

In the postwar period particular emphasis unquestionably will be placed upon "high quality." Dry milks are products whose quality is directly influenced by the quality of the raw material (fluid milk) used in manufacture. Not only will quality be a consideration, but indeed "uniform" quality will be equally as important.

The development of uses and the production of non-fat dry milk solids, dry whole milk, dry buttermilk, dry whey, and other dry milk products are to be regarded in the light of domestic markets as well as possible foreign markets.

Convenience of usage by the consumer can affect market development for dry milk products. This constitutes a real problem for the dry milk producers because of the physical characteristics of these products. (Most of them are very hygroscopic and are subject to rapid flavor and odor deterioration when improperly packaged.)

It is possible that all of the splendid work accomplished by the industry to date will continue to develop in a manifold manner in the future, depending upon how wisely the industry plans its research, quality development,

application and market development, now and in the years immediately preceding the conclusion of the war; recovery from war requirements.

#### IV. MASTITIS

##### A. Mastitis: From the Dairyman's Viewpoint. T. S. SUTTON, *The Ohio State University and The Ohio Agr. Expt. Sta.*

During the past several years significant contributions to the treatment of this serious disease by the use of chemotherapeutic agents have been made. In certain instances the reports of these researches may have given rise to unwarranted hope and a false sense of security on the part of the practical dairyman because the first responsibility in the control of this disease still rests with the man with the cows and will probably continue to do so for a long time in the future. Contributions from the field of Veterinary Medicine have been of significant help; yet without a thorough understanding of the disease and the exercise of careful sanitary procedures and sound management practices on the part of the dairyman himself, there is little hope of ever bringing the disease under control.

In order that the dairy farmer be enabled to control mastitis in his herd, an educational program based on the following fundamentals should be carefully planned and carried to him:

1. An understanding of the internal structure and physiology of the mammary gland. The purpose of this is to make obvious the difficulties encountered in getting rid of the disease once it has gained a foothold.

2. The cause of the disease and the ways in which the infection is usually spread.

3. The predisposing causes which may lower the resistance of the gland to infection.

4. The effects of the disease on the gland and the milk secreted and practical methods of detecting the disease.

5. The effects of the disease on the production of milk and on the quality of dairy products.

6. Sound dairy management and milking practices to control the severity of the disease and the spread of the infection.

The dairyman with a practical working knowledge of these subjects can best utilize the services of the veterinarian in the treatment of the disease.

##### B. Modern Methods of Treating Mastitis. C. S. BRYAN, *Michigan State College, East Lansing, Mich.*

The proper diagnosis must be made to successfully utilize the udder infusion treatments in a program for the control of mastitis. One of the bacteriological tests must be used to determine whether infection is present or absent, and a careful physical examination of the udder must be made to

determine the condition of the udder tissue. Evidence, at hand, indicates that udder infusions are of no value in non-infectious mastitis and in cases of infectious mastitis with marked areas of fibrosis. The proper diagnosis and treatment must be supported by an adequate sanitary program to insure successful control of bovine mastitis.

#### LATIN AMERICAN DAIRYING

##### A. Dairy Farming in Tropical America. R. E. HODGSON, *Bureau of Dairy Industry, Agricultural Research Administration, U. S. D. A., Washington, D. C.*

Studies of the dairy industries of El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia, and Venezuela were made by the U. S. Department of Agriculture at the request of the Coordinator of Inter-American Affairs. These countries are in the tropical zone but the climatic temperature varies with the altitude. Farming is pursued in all altitudes up to about 12,000 feet. Altitudes of 4,000 feet or above appear most favorable for dairying.

Farming is the principal occupation; the major crops being coffee and bananas for export, and corn, rice, sugar cane, and beans for local consumption. The production of food crops is limited because of lack of the most modern cultural methods, infertile, depleted soil, and small acreages under cultivation. Foodstuffs must be imported in many areas.

Cattle raising is an important agricultural pursuit. The cattle population in the 7 countries is about 15 million, or 65 head per 100 people. Most of the cattle are Criollos, descendants of the Spanish cattle introduced by the colonists. They are about the size of the Jersey or Guernsey and vary in color from light yellow to red. They are multiple-purpose animals, raised for milk, beef, and draft. The average milk production is low and the lactation period short. Local production permits a per capita consumption of milk in all forms equivalent to about 1/3 pound daily.

Improvement in the quality of the native cattle has been attempted by crossing and grading with imported Brahman cattle as well as with imported purebred dairy breeds. The Brahman has increased size and improved resistance to tropical conditions but has not materially increased milk production. The purebred dairy breeds have met with varying success. In the hot lowlands under feeding and care not best suited to their needs they have not done well. This failure probably can be attributed more to management factors rather than to their inability to adapt themselves, although they apparently do lack heat tolerance compared to the Criollo or Brahman cattle. In the highlands the purebred dairy breeds do well under proper feeding and management, and grading up the native stock with them offers the best method for rapid improvement. In the hot lowlands improved strains of

resistant Criollos may be the best type, since they probably would produce as much milk as the environmental conditions on the average farm would permit any breed to produce. Dairying should be concentrated in the highlands.

Cattle improvement would be expedited by fencing the pastures and confining the bulls so that breeding could be controlled. More care is needed in selecting breeding animals. Farmers ought to keep breeding and production records and use them in selecting breeding stock. Dairy-herd-improvement programs need to be established by the national departments of agriculture to assist farmers in developing higher producing herds.

Feeding and management conditions on farms must be improved if high yielding cows are to produce efficiently. Cows are maintained on pasture all year; but in the numerous areas where extended dry seasons occur, pastures dry up and because of deficient feed supply the cows lose weight, fall off in production, and go dry. Lowland pasture grasses are of the coarse bunch type while in the highlands the fine-leaved grasses predominate. Progressive farmers supplement pasture with soiling crops such as corn, sorghum, or banana stalks. Silage is becoming more popular and offers the best way of improving the year-round feed supply. Hay is practically unknown but could be used extensively. The feeding of concentrates is limited largely to market-milk areas. Corn, sorghum, rice bran and hulls, coconut meal, cottonseed meal, and sesame meal are the most popular concentrates.

Knowledge of the feeding value of local feeds and existing nutritional deficiencies is limited. Calcium, phosphorus, and iodine deficiencies are suspected in some areas. Vitamin A may be lacking during extended dry periods when pasture is not supplemented with green feed or silage.

Primitive methods of raising calves result in slow growth, late maturity, unthriftiness, and high mortality. Calves should be weaned soon after birth, confined to pens and raised on an adequate ration rather than allowed to run with the cow and nurse at will or allowed to suck as a part of the milking process.

Parasites, particularly the cattle tick, and numerous diseases are prevalent and account for high mortality. Disease control measures are lacking on most farms and the veterinary service is limited or non-existent.

The governments are giving encouragement to dairy development by establishing schools and experiment stations. The U. S. Department of Agriculture is cooperating in this development in some countries by lending leadership and scientists for experiment stations. The Inter-American Institute of Agricultural Sciences, a research and educational institution, is being developed under the auspices of the Pan-American Union. This institution, located in Costa Rica is designed to train students and to conduct research in all phases of the agriculture of the Americas. At present it is supported

by the U. S. Government through a grant of funds from the Coordinator of Inter-American Affairs to the Pan-American Union, but support in the near future will come from the several American Republics that desire to participate financially in this important work. These various educational and experimental organizations will do much to improve agriculture and cattle farming in the areas which they serve. There is need also for further development in agricultural and livestock extension work.

**B. Central American History, People and Industry.** A. C. DAHLBERG, *Cornell University, Ithaca, N. Y.*

An understanding of Central America must be based upon a knowledge of its early history, people and industry. The Indians who inhabited the country when discovered and conquered by Europeans were advanced in the civilization of that time. The Spaniards lead in the conquest and are today the leading European race. They tended to rule and exploit rather than settle and develop. Today the Indian still predominates with a small percentage of Negro and European except in Costa Rica where Europeans constitute the bulk of the population.

Judged by our standards, which is probably not fair but there is no other basis available to us, these countries are relatively poor and undeveloped. This is not entirely a problem of races and climate for there are regions populated by Europeans, and at higher elevations the climate is temperate. These countries are rather small in comparison with all northern neighbors. They need to develop transportation, especially roads and railroads, to avoid excessive isolation in many localities. They need development in many national projects such as public health, schools, agriculture, industry, etc. These things can be done only when sufficient funds can be raised by public taxation to permit the governments to be more active in promoting projects for the common good. The income of the average person needs to be increased to develop a more prosperous middle class. Some home industries together with agricultural advancements would materially assist along these lines. Finally, such developments would give a much greater proportion of the population from which leaders could be selected for election or appointment to the office required by their democratic forms of government.

The United States can be of much assistance to these nations by friendly cooperation over the years to enable Central America to develop itself to the fullest extent. Such a policy is mutually advantageous.

**C. The Dairy Products of Central America.** A. C. DAHLBERG, *Cornell University, Ithaca, N. Y.*

Increased per capita consumption of milk and milk products would improve the nutrition of the people of Central America. The present milk supply available for all purposes varies from 0.2 to 0.7 pounds of milk per person per day.



The three principal dairy products are milk, cheese and butter. Milk is boiled before use and is served chiefly as coffee with hot milk. There are a few good milk-pasteurization plants. These plants might very advantageously make cheese, milk drinks, and ice cream mix. The sanitary quality of the milk is generally poor but there are notable exceptions.

Fresh rennet-curd, highly-salted cheese predominates with some cured soft Muenster type and a little cheddar cheese. The fresh curd cheese probably has 5 per cent salt and is sometimes smoked. The milk is often partially skimmed. The cheese is nearly all made on farms. Cured cheese ought to be more generally manufactured to conserve milk solids from periods of surplus to the dry season. The people are fond of cheese.

The butter is made on farms from raw cream churned about twice weekly. In the higher altitudes some good farm butter is made but in the tropical climate the butter is greasy and spoils quickly without refrigeration. In such areas the production of butterfat should be preferred to butter.

Ice cream production is very restricted but is increasing. There are a few ice cream plants. There is one condensed milk plant. It is doubtful, however, that sufficient milk is available to warrant condensed and dry milk plants.

There is every reason to encourage dairying in the higher altitudes where the excellent grass, good water and temperate climate favor the industry.

**D. Thomas Jefferson in Agriculture.** O. E. REED, *Chief of the Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.*

Last year marked the 200th anniversary of the birth of Thomas Jefferson, the third President of the United States, who was born in Albemarle County, Va., April 13, 1743.

Early in 1943, Congress passed a resolution urging National recognition of the birth of this great American by recalling his many public services. Every school boy and girl knows that Thomas Jefferson wrote the Declaration of Independence. He also served as Governor of Virginia, U. S. Minister to France, Secretary of State, and President of the United States.

Although his life was largely devoted to public service for the Nation he helped create, Jefferson considered himself a farmer. His letters and records indicate his keen interest in agriculture, and in experimental methods and inventions for its improvement. In the field of agriculture he was as much a leader as in the field of statesmanship.

It is entirely fitting and natural, therefore, that this Association pay tribute to Thomas Jefferson, the farmer, the scientist, the extension leader.

Jefferson regarded agriculture as a profession on a level with other professions. He considered agriculture a science and thought that professional scientific people were needed for its development. He felt that colleges

should make provision for training and education in agriculture, the same as in other professions. He also recognized the need for getting scientific agricultural knowledge to farm people. Thus he was among the first of our great leaders to think about the objectives for which our agricultural colleges and experiment stations were eventually founded.

In Jefferson's day, the one great industry of the State of Virginia was agriculture, but this was still of the plantation type, with landholdings in large acreages, and a one-crop system predominating. Tobacco and grains were grown for export, year after year, and eventually tended to deplete the land of its virgin fertility. As a result, the farms quickly became run-down and very much eroded.

In his travels in Europe, as well as in the United States, Jefferson had an opportunity to see many different farming conditions and methods. He felt that pioneering methods in Virginia could be improved. His observations and interest led him to make a test farm out of his mountain-top plantation at Monticello. He planted domestic and imported trees and seeds, and kept minute records to see whether foreign specimens could be adapted to the soil and climate of this country.

He kept a farm book in which he recorded interesting observations about farming, and soil fertility. For example, in his farm book he mentioned a suggestion by John Taylor, an eminent agriculturist of Caroline County, Va., about the use of cattle manure. The quotation from his farm book is as follows:

"Mr. Taylor says he knows by accurate and constant experience that 40 head of cattle folded of nights only, dung completely 20 yards square. Before folding, the ground should be coultured and covered with straw, then folded one week and the straw and dung immediately turned in with the great plough."

Then Jefferson himself recorded an experiment to be tried, comparing a mixture of cow manure and sheep manure applied by the "folding" method, with a field where direct application of manure was made without "folding." He also wrote that rotted manure should be applied in December, January, or February at the rate of 25 loads per acre, and that it would be well worthwhile to confine and litter cattle in a yard through the summer—each head would then manure an acre a year.

During Jefferson's first long absence from Monticello in the public service (approximately from 1784 to 1794), his fields suffered from neglect under overseers' management. When he returned in 1794 he worked out a system of crop rotation to restore fertility. He divided his farm into six fields to be put under this rotation: First year, wheat; second, corn, potatoes, and peas; third, rye or wheat; fourth and fifth, clover; sixth, folding and buckwheat dressing.

He was also aware of soil erosion and had a suggestion for its control, as is indicated by the following letter he wrote in 1817:

"A method of ploughing over hill sides horizontally, introduced into the most hilly part of our country by Colo. T. M. Randolph, my son in law, may be worth mentioning to you. He has practiced it a dozen or 15 years, and it's advantages were so immediately observed that it has already become very general, and has entirely changed and renovated the face of our country. Every rain, before that, while it gave a temporary refreshment, did permanent evil by carrying off our soil: and fields were no sooner cleared than wasted. At present we may say that we lose none of our soil, the rain not absorbed in the moment of it's fall being retained in the hollows between the beds until it can be absorbed."

He was much interested in all kinds of crops, both garden and field, but particularly soil-improvement crops, such as clovers and other legumes. The latter he seemed to value more for their soil-improving properties than for feeding livestock.

Most of Jefferson's interest in livestock seems to have been centered in sheep and dogs. He had draft animals to be sure and some of these were oxen. He must have had milk cows to furnish milk, butter, and cheese for his many farm households, but we find nothing in his letters or reports to indicate what breed they may have been or how much milk they may have produced.

His special interest in sheep is indicated in a letter to President Madison, who was also a sheep enthusiast. He suggested that they give all their full-blooded males to the different counties throughout the State, as fast as they could furnish them. He thought this would encourage the formation in each county of small societies to maintain and provide rules for the use of the rams. That would seem to indicate that Jefferson was thinking about something similar to our present-day breeding circuits.

Jefferson's interest in dairy matters is also apparent in his notes and letters. In his account of a journey from Paris to the southern parts of France and northern Italy in 1787, he devotes considerable space to his observations on the making of butter and cheese. Years later in a letter to the editor of the *American Farmer* he recounted some of his observations on the making of Parmesan cheese at a dairy at Rozzano, which he says he "attended from sun-rise to sun-set, made short notes on the spot of what was passing under my eye, and when I returned to my lodgings at Milan at night, I wrote them at full length."

There in the stables he "saw 85 cows, fed on hay and grass, not on grain." He wrote that the milk was "scummed" and that butter was made from the cream, and cheese from the scummed milk. He recorded the day's operation in great detail, even such details as the fact that a quarter of an ounce of saffron was used to give color to the cheese.

When the cheese was made, the whey was put back in the kettle and the buttermilk was added also. From this they made a poor cheese for the

country people, and the whey from this was given to the hogs. Eight men, he said, "suffice to keep the cows and do all the business of the dairy."

I mention this to show that Jefferson after all was quite interested in dairying and no doubt had home-made cheese and butter in his own larder throughout the year.

But perhaps Jefferson's most famous dairy experience was in connection with the mammoth cheese that was presented to him at the White House on New Year's Day, 1802, by Elder John Leland of Massachusetts.

Elder John Leland was born and grew up in Massachusetts, but in 1778 he moved to Orange County, Va., not far distant from Jefferson's home. A strong friendship was formed between the two men. After 14 years in Virginia Leland returned to Massachusetts with his family and settled in an agricultural community near Cheshire.

Here Elder Leland helped by sermon and otherwise to promote the general acceptance of Jeffersonian principles and when Jefferson was elected President, Leland conceived a plan of celebrating his hero's election by presenting him with a cheese made from the milk of every cow within the precincts of Cheshire. On a specified day, July 20, 1801, curd was brought in from all the Cheshire farmers (excluding the Federalists) and a cheese was made that was 4 feet in diameter and 18 inches high, and that weighed 1,235 pounds. A cider mill was used for a press. The cheese was in the press 11 days, after which it was stored in a curing room until ready to be shipped to Washington.

In accepting the gift President Jefferson stated that he looked upon this New Year's gift as a token of the fidelity of the people of the country to the great cause of equal rights to all men, that he would cause the event to be placed upon the archives of the Nation, and that he would ever esteem it as one of the happiest occasions of his life.

The news of this event spread throughout the country and Elder John Leland became more famous than ever. A certain Federalist is said to have asked Elder: "Is it true that President Jefferson found some skippers in the cheese when he cut it?" The reply was, "It is quite possible, for although we tried to exclude all Federalists we learned afterwards that one or two got by us and some of their curds may have contaminated the cheese."

If Jefferson were alive today, I have no doubt but that he would be the owner of a herd of well-bred, registered dairy cattle. He would have the best of equipment on his dairy farm and would practice the kind of dairy husbandry that would lead to improvement in the efficiency of his animals, in the fertility of his fields, and the production of farm products of the highest quality. The records of his interests and achievements all point in that direction. He was also interested in forming agricultural societies, for mutual aid and study of technical problems. It is my feeling, therefore, that he would heartily approve of the work our American Dairy Science Association is doing and might even be a member of the Association.

## EXTENSION SECTION

**E1. Dairy Herd Improvement Association Organization and Agreement Forms.** W. T. CRANDALL, *Cornell University, Ithaca, N. Y.*

Early in this war it was apparent in New York that counties were most successful in maintaining D. H. I. A. work when their testing work was organized on a county wide or district basis. Some counties with independently organized associations found it desirable to reorganize under the centralized plan in order to: (1) Rearrange routes to reduce travel of supervisors. (2) Arrange for smaller circuits to be tested by part-time supervisors. (3) Shift supervisors from one circuit to another to prevent the loss of two consecutive months' records in any one herd. (4) Combine any circuit losing its supervisor, with some other circuit for bi-monthly testing until a new supervisor can be obtained. (5) Give greater consideration to herds carrying on long-time breeding programs and H. I. R. tests.

It is important in a centrally organized county or district association that directors be chosen so that each circuit will have active representation. Each circuit should maintain separate identity in the reporting and summarization of its records.

It is suggested that any group of D. H. I. A. members be considered a circuit when it tests regularly with a full-time supervisor; or when it tests with a part-time supervisor with less than a full membership either because of geographical location, inability to get a full membership or the use of one or more supervisors who can devote only part time to association testing.

Agreements outlining the responsibilities of dairy herd improvement association members and supervisors in carrying on the business and work of D. H. I. A. testing are not legally binding and should not use legal and formal language. Such agreements are worth while in outlining the obligations and full responsibilities of the supervisor, the members and the organization.

Agreements should be simple but definite in statement of duties, so that there can be no misunderstanding on the part of either the supervisor, the member or the officers as to the purpose of the work or as to what must be done to accomplish that purpose.

**E2. Short Cuts in Record Keeping.** J. F. KENDRICK, *Dairy Bureau, U. S. Department of Agriculture, Washington, D. C.*

The present method of obtaining, calculating, and recording feed and production records in dairy herd improvement associations involves a great amount of detail record work, and in some instances duplication of work, to obtain data now required in the complete D. H. I. A. program. Perhaps some of the detailed work and most of the duplication of work may be reduced or eliminated without affecting the reliability of the records or their usability in the D. H. I. A. program.

With manpower at a premium, it is appropriate that the record-keeping methods of dairy herd improvement associations be critically analyzed to determine if the methods now in use can be streamlined to simplify the record-keeping work and thereby reduce the time required of association supervisors to obtain satisfactory records.

Some States have devised new methods of calculating production records and others have developed simplified record-keeping forms, all conforming with the basic rules governing association testing, which reduce the time required for the association supervisor to obtain and record satisfactory data. These developments should be reviewed and studied to determine if any of the methods developed should be adopted generally.

The record-keeping forms now in general use in associations have evolved through periodic revision of old forms to serve new phases of association work as they were developed and added to the program. It is appropriate to review as a whole all record-keeping forms now in use and explore the possibility of developing entirely new and simplified forms incorporating all the improved features that have been developed that are sound and practical for general use to serve the overall dairy herd improvement program as it is now conducted.

#### E4. Securing Supervisors During and Immediately Following the War. C. R. GEARHART, *Pennsylvania State College, State College, Pa.*

The securing of D. H. I. A. supervisors seems to be as difficult, if not more so than it was in the fall of 1942. This is due to the drive made by Selective Service for more men in the Armed Forces. Practically no deferments for men under 26 and very few for those over 26 are being granted. Our difficulties are increased by Selective Service rescinding "Activity and Occupational" Bulletin No. 18, which listed D. H. I. A. supervisors among critical occupations. They are, however, still listed among essential occupations in "Activities and Occupational" Bulletin No. 5.

Many Draft Boards are somewhat confused as to their right to defer supervisors even though they feel that such action is justified. Deferring supervisors does not always solve our problem. Over half of the men that are deferred cannot stand the remarks, and insinuations that are often made, and enlist in the Armed Forces. The securing of cars, tires, and gasoline add to the difficulties of securing supervisors, but are minor factors compared to the loss of supervisors to the Armed Forces.

With these facts in mind, I shall review past methods used in securing supervisors, and recommend one or two new ones. In Volume 25, No. 8, issue of *Journal of Dairy Science* are a few suggestions made in 1942, namely, "Helping Qualified Tester Personnel," by A. J. Cramer, "Superior and Problems," by H. E. Loveland, "Emergency Adjustment in D. H. I. Procedure," by C. R. Gearhart, "Current Developments Affect-

ing D. H. I. A. Work," by Joseph B. Parker. The key thought in all of these briefs is the securing and maintaining of D. H. I. A. supervisors.

Some suggestions were made that did not prove practical, such as the use of local dairymen, 4-H Club members and Vocational Agriculture students who had not taken a short course, to keep the work going until a regular tester could be secured.

In 1943, Volume 26, No. 8 issue of the JOURNAL OF DAIRY SCIENCE, we again find four papers, the central thought of which is ways and means of keeping D. H. I. A. work going after the regular supervisor leaves. Page 719—"The Use of Conscientious Objectors as Dairy Herd Improvement Association Supervisors," by C. R. Gearhart. Page 721—"Vocational Agriculture and Junior D. H. I. Associations in War-time D. H. I. A. Adjustments," by R. F. Evans. Page 722—"Bi-Monthly Testing as a War-time D. H. I. A. Adjustment," by E. C. Scheidenhelm. Page 723—"Combining Associations," by Floyd J. Arnold.

*Securing and holding supervisors.* This past year, every effort was made to locate and train qualified men and women for D. H. I. A. positions.

Short courses were given every two months. We have used newspaper articles, radio talks, and personal letters to locate testers.

Special notices were sent to county agricultural agents and vocational agricultural teachers.

The Draft Boards have assisted in locating men placed in 4-F and yet we have less than a dozen 4-F supervisors in the field.

The State U. S. Employment office has offered their assistance, and two supervisors from that source are now in the field.

Our attempts to interest men over draft age have been rather unsuccessful. We have secured only three such men during the past two years. The change of beds and diet seems to be the main objection of men in the over-age group.

We have fourteen girls working now, with only one or two prospects for getting more. We are well pleased with their work. It is usually neater and just as accurate as that done by the men. Since December, 1941, there were 23 girls hired by associations, nine of whom have resigned. Moving to different farms over some roads that are not too good, and in all kinds of weather is the main objection of the average girl.

A number of our testers are under draft age, but it is too much to expect these boys to conduct the work as it should be done. All they can do is keep the most fundamental records.

We have forty-one conscientious objectors, who are doing excellent work. Most C. P. S. men take an interest in their work even though they receive no financial reimbursement. They are courteous and trustworthy, and are respected by their dairymen. Of the forty-one, I do not know of one that uses tobacco, liquor or profane language. As one dairyman put it, "We

appreciate the absence of profanity, unclean stories, etc., which might influence our children."

During 1943, we were able to keep the old associations supplied with testers practically 100 per cent. In only a few cases was it necessary to resort to cooperative testing, that is one association loaning their supervisor—one month at a time—to neighboring associations. In a few cases, the association losing their supervisor was allowed to miss one month and that month averaged by using the preceding and succeeding months' records.

*New source of supervisors.* The only new source of securing supervisors is discharged soldiers.

Work was started on this nine months ago, with a willingness to cooperate all the way from Washington, D. C., down to the county organizations. Since starting on this project, personnel and channels have been changed, making it difficult to know just what contact to make.

*A hoped for plan.* To make the project practical, I feel there should be one person who has contact with soldiers as they are being discharged and who knows about the need and qualifications of supervisors. When he finds men interested, he can direct them to get in touch with the county agricultural agent located in his home county.

He should be in a position to put out information relative to the needs and qualifications of supervisors. When he finds a soldier interested, check on his qualifications, and keep in touch with him until he has been discharged and arrangements have been made for his attending a D. H. I. A. Short Course.

*Present plan.* At present we are keeping in touch with State Headquarters of Selective Service Re-employment Division; The Veterans Employment Division; and the U. S. Employment Service; all located at Harrisburg, Pennsylvania, the State Capital.

Through the State U. S. Employment Service, we have sent a letter to the 91 local offices, scattered throughout the state. This letter stated the qualifications of a supervisor and requested that the local representative of the U. S. Employment Service get in touch with the local county agricultural agent, when finding a prospect.

So far we have obtained only 2 discharged soldiers.

#### **E5. Streamlined Testing for Dairymen.** *Department of Dairy Husbandry, University of Wisconsin, Madison, Wis.*

In 1941 a study was made of D. H. I. A. programs in California and Washington to ascertain the adaptability of central laboratory testing to Wisconsin conditions. Two county-wide cooperative associations were organized that year. The advantages of this system of testing soon led to the organization of other associations. Under the conditions imposed by the



war, great development of this testing program is under way in this state. At present 28 County Cooperative D. H. I. A.'s are organized or in operation.

The principal advantages of the program are: (1) Provides any type of testing service desired by any dairyman in the county—A. R., H. I. R., D. H. I. A. Standard, or Owner-Sampler. (2) Permits testing 10 to 20 per cent of all cows in any county at all times. (3) Provides sufficient income to pay testers much more satisfactory salaries, plus travel. (4) Central laboratory facilities contribute toward more accurate records with less inconvenience to herd owners. (5) Program is conducted as a business governed by the Cooperative Laws of the state.

**E6. Testing in Areas of Small Herds and Scarce Membership. R. A. CAVE, *South Dakota State College, Brookings, S. Dak.***

South Dakota represents an area as outlined in the topic for discussion. Since the writer is familiar with the dairy herd improvement work in that state he can best discuss the subject by describing the progress made.

The operation of dairy herd improvement associations in South Dakota has fluctuated widely since the first one was started in 1923. From that time until 1931 the number of associations increased to 14 with 340 herds and 5000 cows on test. This was a period of fairly good crops and relatively high prices for dairy products as compared to the prices of meat animals and feed crops. Interest in dairying was increasing and many dairy heifers were imported from Wisconsin and Minnesota for 4-H dairy club members.

The depression and several years of severe drought in the early thirties was disastrous to all livestock production and by the spring of 1936 had reduced the volume of testing to two associations containing 18 herds and 440 cows. In spite of the relatively unfavorable prices for dairy products as compared to meat animals since 1936, the associations were increased to eight with 150 herds and about 3000 cows by 1941. In 1942 and '43 all of the supervisors were either deferred for agriculture or inducted into the armed forces.

At the present time we have three associations containing about 45 herds, operated by two supervisors. One is 60 years old and the other 70. They are both interested in their jobs and are doing satisfactory work. They tend to business and do not run around nights. The 60-year-old supervisor operates one association of 18 members on a monthly basis and another of 13 members on a bi-monthly basis. We have not attempted to hire women or girls as supervisors because of the long distances to travel and the sentiment against it.

Continuous operation of dairy herd improvement associations in South Dakota is confronted with many obstacles.

There is a lack of interest because of a cash grain farming background and an antipathy toward milking cows and doing chores.

Large farms averaging about 500 acres cause crop raising and livestock, other than dairy, to compete very strongly with the dairy enterprise for the time and attention of the farm operator.

There is much shifting back and forth between dairying and meat animal production as the relative prices for those products change.

The number of cows per head has averaged about 14 since the associations were first started.

Cows are milked and cream or milk sold from about 85 per cent of the farms in the state. The dairy enterprise, however, is secondary on a large per cent of the farms and the owners who desire to join a dairy herd improvement association are few and far between.

It is often necessary to combine the interested dairymen of three or four counties in order to have a sufficient number to make a full association. The supervisor's route sometimes covers 300 miles or more. The holding of meetings, to attend to the business of the association and discuss dairy problems, is very difficult. It is also hard to maintain membership so the supervisor will have full-time work.

Whenever a group of dairymen is interested in organizing a D. H. I. A. they are given all the assistance possible by the extension dairyman and county agricultural agent. The cooperative creamery or other marketing agency is usually interested and will help compile a list of dairymen as prospective members. These dairymen are contacted personally, by letter, or at called meetings. A personal interview, by an interested dairyman, the county agricultural agent, or the county agricultural agent and extension dairyman, is usually most successful in obtaining members. Excuses for not joining are legion but when analyzed usually mean that the herd owner objects to an action program.

If enough members for a full association cannot be obtained in one county, dairymen in adjacent counties are solicited. A meeting is then called by the county agricultural agent for organization. At this meeting a board of directors is elected which selects its own officers consisting of president, vice-president and secretary-treasurer.

The fees for testing are agreed upon and consist of a flat rate for herds of 10 cows or less plus 10 cents per cow above 10.

Candidates for the position of supervisor are presented, when possible, by the extension dairyman and selected by a vote of the directors. The supervisor is given several days of training at the state college or trained right on the job by the extension dairyman.

Supervisors are paid by each member at the time each herd is tested. The amount of his pay depends upon the number and size of the herds in the association. In some instances monthly dues of 10 cents per member

are collected by the supervisor and turned over to the treasurer of the association. After the supervisor becomes acquainted, he assumes some responsibility in keeping up the membership.

The membership agreements provide for one year of testing. They contain a clause requiring members, who withdraw during the year, to pay the testing fees for the entire year or get a substitute who is satisfactory to the board of directors. No attempt has ever been made to enforce this provision in the agreement. When members drop out, thus, cutting down on the income as well as the time of the supervisor; the county agricultural agent, officers, supervisor, and sometimes the extension dairyman make every effort to get new members. Small associations with part-time supervisors have not been very satisfactory as the testing is likely to be irregular. In one instance the advanced registry work at two state institutions was combined with eight regular D. H. I. A. herds and worked out quite satisfactorily.

After an association has been in operation a few years new members have joined at irregular intervals and the close of the testing year is not the same for all herds. Since there is no date for stopping operation without leaving some herds with incomplete yearly records, the tendency is toward continuous operation of the association except when the supervisor is lost. Annual meetings are held if possible to elect officers, discuss dairy problems, get acquainted and plan the program for the year.

A summary of the herd records is compiled each month from the supervisor's monthly report to the extension dairyman. This summary also contains news items and timely discussions of subject matter. It is mailed to each member, and to a mailing list of former members and county agents, under stamp.

In South Dakota the dairy herd improvement association is looked upon as the most effective means of raising the efficiency of the dairy herds. Every effort will be made to keep them alive and functioning to the fullest extent possible.

**E7. Wartime 4-H Dairy Club Program.** J. C. NAGEOTTE, *Pennsylvania State College, State College, Pa.*

Before the war our dairy 4-H program included the careful selection of calves, group meetings at which we gave instructions in feeding, fitting, and management. This was followed by a round-up at the end of the year when all the calves were exhibited and the club members were scored on the type, fitting, showing, and record book of their project.

War restrictions on travel, and shortage of farm labor made it seem too impractical to carry on the former program. We were forced to decide whether we should curtail our program; or change our method of conducting this project. Increased demand for calf club work helped us to decide to modify our method of conducting the work.

Instructions on care and management had been incorporated in the club record book, and by frequent new editions we have been able to keep this up to date. This made it possible to eliminate part of the meetings.

The selection of the calves we have left in the hands of the parents and local county agents. The quality of calves hasn't been too bad as a result of this practice; but we feel this change has not been for the best. More time is needed to see the results in the selection of calves.

The most drastic change was the elimination of the round-up at the close of the club year as a means of measuring the quality of the club work. We have substituted for this a scoring system which is designed to measure how effectively the club members apply the instructions given them on feeding and care of calves. This scoring is done in the club member's barn.

There are two score sheets used, one for first-year members, and one for second-year members. (Copies of these sheets will be available for distribution.) The first-year score is based on the selection of the calf, breeding and type, the feeding program, housing and management, training and fitting, record books, and attendance at club meetings.

The second-year score includes the following headings: The breeding program followed, type, feeding, stabling, growth, training and fitting, record books, and attendance at club meetings.

Club members receiving a score of 90 points or more receive a blue ribbon; 80 to 89, a red ribbon; 70 to 79, a white ribbon. Any receiving a score below 70 are awarded no ribbons, but are credited with a completion.

The scoring is done by a Dairy Specialist or a county agricultural agent. We find that fewer blue ribbons are awarded than were at the round-ups. A few scores went over 100, but most fall between 70 and 89. There were as many below 70 as there were over 100. We also found we were scoring the parents as much or more than we were the club members. The parents found this out too, and the results have been most encouraging in getting a good calf-raising program established on these farms. If calf-club work is to be a worthwhile project it must improve practices of our farms. This scoring system has decidedly helped do this.

The trend this year is to have more calf-club shows, not round-ups; with the farm score used to measure the quality of the club work and the award to be given. We doubt if we will give up the scoring system and go back to the old type round-up after the war. However, we feel that a calf-club show for those who are interested in showing is necessary; but we should have some other form of completing the project for those who do not care to, or cannot show.

**E8. The Forced Ventilation System of Curing Hay in the Mow.** R. G. CONNELLY, *Virginia Polytechnic Institute, Blacksburg, Va.*

The types of forage plants, the cultural practices followed in their production, and the methods used in harvesting, curing and storing hay crops

largely determine the feeding qualities and milk production value of the hay. The forced ventilation system of curing hay offers several possibilities as a practical method for saving hay crops, for conserving practically all the plant leaves and a large percentage of green color; and for providing a more palatable hay of higher protein content for dairy cattle.

The "Mow Hay Drier" consists of a series of air ducts built on the floor of the hay mow. It is designed so that a large volume of air, under low pressure (at least 10 cu. ft. per minute) can be distributed uniformly throughout the duct system. The air pressure is maintained by a motor-driven, multi-vane fan. The single system has a central air duct that tapers from the fan housing at one end of the mow, down the center line on the mow floor to the opposite end of the mow. Smaller lateral ducts, spaced at 4- to 5-foot intervals, extend at right angles from both sides of the main central duct, to within 5 feet of the walls of the mow. The central duct is sealed, except for the vents that lead into the lateral ducts, which resemble inverted wood troughs, blocked up one inch from the mow floor to permit the uniform escape of air into the hay deposited on the duct system.

Hay cured properly in the mow by forced ventilation is superior to hay from the same cutting cured in the field.

When samples of mow- and field-cured hay from the same cutting were inspected by U. S. Department of Agriculture hay grades, the mow-cured samples were graded consistently one to two grades higher than the field-cured hay.

Samples of mow-cured alfalfa hay, leafy, green, showing less than  $\frac{1}{4}$  bloom, from the 1943 crop, taken in March and April, 1944, from mows on five different Virginia dairy herd improvement association farms, analyzed 92 to 93 per cent dry matter; 3.23 to 3.75 per cent nitrogen, or 20.19 to 23.44 per cent crude protein on a calculated basis. One especially leafy, green sample of mow-cured alfalfa hay, still showing purple color in the bloom, was taken from the mow on a sixth Virginia dairy farm. This sample analyzed 92 per cent dry matter; 4.39 per cent nitrogen, and 27.44 per cent crude protein on a calculated basis.

The alfalfa hay cured by forced ventilation on the above five Virginia dairy herd improvement association farms was fed to the respective farm dairy herds. Under the practical conditions maintained on these farms, the average monthly milk production per cow for the five- to seven-month period when mow-cured hay was fed was consistently greater than the average monthly milk production per cow for the comparable five to seven months of the previous year when field-cured hay was fed.

The cows in Herd No. I averaged 536 pounds of milk per month on field-cured hay in the 1941 hay-feeding period, and 644 pounds of milk, and 615 pounds of milk per month on mow-cured hay in the comparable 1942 and 1943 hay-feeding periods, respectively. In Herd No. II the cows averaged

676 pounds of milk per month on field-cured hay in 1941; fed mow-cured hay they averaged 681 pounds, and 698 pounds milk production in 1942 and 1943, respectively. The average monthly milk production per cow in Herd No. III was 570 pounds for the months in 1941 when the herd was fed field-cured hay; and 623 pounds, and 610 pounds per month for the comparable periods in which they were fed mow-cured hay in 1942 and 1943, respectively. In Herd No. IV the cows averaged 673 pounds of milk per month on field-cured hay in 1942, and 757 pounds of milk on mow-cured hay in 1943. The cows in Herd No. V averaged 909 pounds of milk on field-cured hay in 1942, and 1040 pounds per cow on mow-cured hay during the comparable hay-feeding period of 1943. All herds were milked twice daily. Silage was fed with the hay. The grain supplement, containing approximately 13 per cent crude protein in Herd No. V, and 14 to 18 per cent crude protein in the other four herds, was fed by weight according to milk production.

**E11. Action Program for Rapid Washing Method.** J. M. JENSEN, *Michigan State College, East Lansing, Mich.*

Considerable attention has been given to a field program involving practical rapid methods of washing dairy utensils and utilizing wetting agents and sodium hexametaphosphate as detergents.

The rapid "flush" methods of washing separators and milking machines were tried and found to be highly satisfactory, after which the procedure was presented to inspectors, sanitarians and county agricultural agents before being taken to the field in form of demonstration meetings.

Unusual interest was shown by audiences in these demonstrations. As a result of 32 meetings attended by 1,300 persons more than 100,000 pounds of wetting agent detergent alone was reported sold in the first six months after its initiation in lower Michigan.

## PRODUCTION SECTION

**P1. Some Factors Affecting the Nutritive Value of Korean Lespedeza Hay.\*** H. A. HERMAN, E. W. SWANSON AND A. C. RAGSDALE, *University of Missouri, Columbia, Mo.*

Digestion trials with growing dairy heifers have indicated a wide variation in the nutritive value of Korean lespedeza hays. Routine chemical analyses have been found to give a poor index of the nutritive value of the hay because of the characteristic tendency of Korean lespedeza to contain large amounts of lignin. This plant generally contains about 50 per cent more lignin than alfalfa. Typical samples were found to contain 17 per cent lignin in the stems and 23 per cent in the leaves, whereas in alfalfa the stems are about twice as high in lignin as the leaves.

\* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 950.

Digestion trials with 12 growing Holstein heifers fed Korean lespedeza cut at various stages of growth indicate a decrease in total digestible nutrients with increasing lignin content and advancing maturity of the plant. Korean lespedeza cut for hay at an early to intermediate stage of growth contained 52.03 per cent total digestible nutrients, whereas the late-cut hay contained an average of only 37.9 per cent. Early-cut hay contained about 15 per cent and late-cut from 19.9 to 23 per cent lignin. Holstein heifers weighing an average of 800 pounds, and fed 2 pounds daily of the late-cut lespedeza hay per hundredweight, were unable to make gains in nitrogen storage and were occasionally in a negative balance. Since we have found mature, highly lignified, Korean lespedeza hay to be of lower nutritive value, it tends to further explain the deleterious effects on milk production where dairy cows are forced to consume large quantities of such hays. Early-cut or immature Korean lespedeza is comparable in nutrient content to alfalfa hay, but unfortunately much of it is harvested at a stage too mature for good quality hay of highest feed value.

**P2. The Feeding Value of Cheat for Dairy Cows.\*** M. H. BERRY, K. L. TURK, AND L. A. MOORE, *University of Maryland, College Park, Md.*

Two feeding trials were conducted for the purpose of determining the feeding value of ground cheat (*Bromus secalinus*) as compared with ground barley when fed as 30 per cent of a grain ration for milking cows. In Trial I, two groups of six milking cows were fed on a double reversal trial for three six-week periods. In the second trial, two groups of five milking cows were fed on a double reversal trial for two six-week periods and for one four-week period because an insufficient amount of cheat was available to carry the cows to the end of the regular period.

The only variant in the ration fed to both groups in both trials was the substitution of cheat for an equal amount of barley in the grain mixture. Grain was fed according to milk production, allowing one pound for every three pounds of 4 per cent fat-corrected milk. Alfalfa hay was fed at the rate of one pound per day and corn silage at the rate of three pounds per day for each 100 pounds of live weight.

In the first trial, the cows on the cheat mixture averaged 27.2 pounds of milk and 1.3 pounds of butterfat daily with an average per cent fat of 4.8. The cows fed the barley mixture averaged 26.7 pounds of milk and 1.3 pounds of butterfat daily with an average per cent fat of 4.7. When the milk produced by each group was converted to 4 per cent fat-corrected milk, the cows on the cheat mixture averaged 30.1 pounds and the cows on the check or barley ration averaged 29.6 pounds daily.

There was no significant difference in the feed consumption of the two groups. The average change in live weight per cow per week for the trial

\* Scientific Article A85a, Contribution No. 1942 of the Maryland Agricultural Experiment Station (Department of Dairy Husbandry).

was also insignificant. The cows on the barley mixture lost an average of 1.2 pounds and those on the cheat mixture lost an average of 2.2 pounds per cow per week for the entire experimental period.

In Trial II, approximately the same results were secured as were in Trial I. The cows fed the cheat mixture averaged 25.9 pounds of milk and 1.1 pounds of butterfat daily with an average per cent fat of 4.3. The cows fed the barley mixture averaged 25.2 pounds of milk and 1.2 pounds of butterfat daily with an average per cent fat of 4.8. When the milk produced by each group was equated to its equivalent value in 4.0 per cent fat-corrected milk, the cows on the cheat mixture averaged 27.4 pounds of milk and the cows on the barley ration, 28.1 pounds of milk daily.

In this trial as in Trial I, there was no significant difference in the feed consumption of the two groups. In Trial II, the cows on the cheat mixture maintained their weight at a very slightly higher level throughout the trial. The cows on the cheat mixture made an average weekly gain of 4.6 pounds per cow and those on the barley mixture 4.1 pounds per cow per week.

The average composition of the cheat fed in these trials was as follows: 8.60 per cent water, 12.36 per cent protein, 2.13 per cent fat, 8.14 per cent fiber, 2.95 per cent ash, and 65.84 per cent nitrogen-free extract.

The average composition of the barley fed in these trials was as follows: 10.95 per cent water, 12.75 per cent protein, 2.24 per cent fat, 5.33 per cent fiber, 2.30 per cent ash, and 66.45 per cent nitrogen-free extract.

The results obtained in these two feeding trials with dairy cows show cheat to be equal to barley in feeding value for milk production. Chemical analyses of the cheat and barley fed in both trials showed cheat to be practically the same as barley in composition.

### P3. Invisible or Fermentation Losses in Silage Making. A. E. PERKINS, *Ohio Agricultural Experiment Station, Wooster, Ohio.*

Certain losses encountered in silage making, such as leakage of juice or top spoilage, are obvious and with ordinary methods may be determined if desired. These losses, moreover, may with proper care be largely or entirely avoided.

Another type of loss which occurs in the ensiled mass cannot be so readily detected or avoided. Water and gases are formed as by-products of chemical or fermentative reactions, some of which escape or are of no further nutritive value. The exact extent of this type of loss is not well known and is not readily measurable. The total loss of weight of the ensiled material when evaporation is avoided gives a rough measure of the "Fermentation loss."

Work with a series of small experimental silos is reported in which the invisible or fermentation loss is determined by the "total loss of weight" method. One series of 49 such observations previously reported gave an



average loss of 4.18 per cent. The loss seemed to be greater with some crops than with others. A series of 62 observations during the 1943 season gave an average loss of 3.56 per cent. Both these values are probably too high because of imperfectly controlled evaporation.

These values are, nevertheless, in decided contrast to those in the neighborhood of 15–20 per cent which have often been reported, especially when procedures which involve the drying of samples of the silage are employed. A parallel series of water determinations on 68 samples of silage by oven drying and by a distillation technic showed an average difference of 2.05 per cent on the fresh basis, equivalent to 8.95 per cent dry basis. Dry matter, if taken as 100 per cent minus the water, would vary to the same extent in reverse order of magnitude.

This would account for a major part of the discrepancy in results between ensiling losses obtained by the total-weight-loss method and others obtained by procedures which involve oven drying of silage samples.

**P5. Effect of Feeding Corn and Alfalfa Silages on the Fat-and-Water-Soluble Vitamins of Winter Milk.** J. J. STEFANIAK, I. W. RUPEL, AND W. H. PETERSON, *University of Wisconsin, Madison, Wis.*

*Carotene and vitamin A.* An experiment to determine whether a corn silage high in carotene would approach an alfalfa silage in maintaining a high level of carotene and vitamin A in the milk was conducted with 3 lots of milking cows. Lot I received corn silage; lot II, equal amounts of corn and alfalfa silages; and lot III, alfalfa silage. Besides silage, all lots were fed a good grade of alfalfa hay and a grain ration adjusted to correspond to the butter-fat production. Each lot consisted of five cows selected so that lactation and butter-fat production were approximately equal. The feeding period began on November 10th and ended on February 10th.

Milk production for the three lots at the beginning of the trial and at the end of the 8th week was as follows (lbs. 4% milk per cow daily): lot I, 29.8, 24.7; lot II, 29.8, 22.1; lot III, 29.2, 22.5.

While the carotene and vitamin A contents of the milks increased with the intake of carotene in the ration, this increase was not directly proportional to the intake. From silage, lot I received 289 mg. of carotene per cow per day, lot II, 664 mg., and lot III 1,039 mg. From the alfalfa hay, about 125 mg. additional carotene per cow per day was ingested. Carotene and vitamin A of the milk expressed as micrograms per gm. of butterfat were: lot I, 4.58 carotene and 5.56 vitamin A; lot II, 5.39 and 5.89; lot III, 5.74 and 6.94. These data show that the corn silage was almost equal to the alfalfa silage in maintaining the carotene and vitamin A contents of the milk.

*Three B vitamins.* During two preceding years, the effect of feeding several different types of silage for about 10 weeks on the contents of panto-

thenic acid, niacin and biotin of the milk was tested. The average figures per ml. of milk were as follows: corn silage, 3.8 micrograms pantothenic acid, 0.69 micrograms niacin and 28 millimicrograms biotin; corresponding figures for soybean-sorghum silage were 4.9, 0.65 and 46; the values for alfalfa silage were 4.1, 0.60 and 49.

Biotin was the only one of the three vitamins which varied significantly with the forage fed. Milk from cows fed soybean-sorghum and alfalfa silages contained about 50 per cent more biotin than that from corn silage.

**P6. A New Feeding Standard for Milk Production.** T. A. BAKER, *University of Delaware, Newark, Del.*

At the Delaware Agricultural Experiment Station five groups of Holstein cows were fed for two consecutive lactations at different levels of total digestible nutrients. The actual levels above maintenance requirements as calculated at the end of the experiment were 82.2, 98.0, 105.3, 122.4, and 131.0 per cent of the Haecker standard.

The addition of a pound of T.D.N. gave as great an increase in milk production at the higher levels as at the lower. The average return from the addition of a pound of T.D.N. to the ration was 1.46 lbs. of 4 per cent fat-corrected milk.

It is recommended that the feeding standard for milk production be raised 20 per cent above the Haecker standard.

**P7. Limited-Grain Ration vs. All-Roughage Ration for Dairy Cattle.** F. B. WOLBERG, A. A. SPIELMAN, V. L. MILLER, AND U. S. ASHWORTH, *State College of Washington, Pullman, Wash.*

At one year of age one group of Holstein heifers was placed on an all-roughage ration consisting of pasture, hay and silage. The other heifers receive similar roughage plus a limited amount of grain, 1.5 to 2 pounds daily during winter only. All the animals are kept in the herd throughout their productive life. During lactation the limited-grain group receives one pound of grain for each eight pounds of milk produced.

Data are presented on the first eight years of this study. Thirty-five heifers in the limited-grain group and 28 in the all-roughage group have been raised from one year of age to calving. The average age at calving was 29.8 months for the limited-grain group, and 30 months for the all-roughage group. The average total digestible nutrients consumption per 100 pounds gain in live weight for heifers not receiving grain was 1386 pounds, and for heifers receiving grain, 1309 pounds. The average gain in height and body weight during a period of 540 days was 16.9 centimeters and 491.8 pounds for the all-roughage heifers.

The number of completed lactations to date for each group is as follows: limited-grain group, first lactation 23, second lactation 13, third lactation 11,

fourth lactation 5; all-roughage group, first lactation 24, second lactation 15, third lactation 10, fourth lactation 4. The average production of FCM was 10,444 pounds for the all-roughage group and 7,941 pounds for the limited-grain group. The gross efficiency of the two groups as indicated by the FCM/TDN ratio is in favor of the grain group with a ratio of 1.47 as compared to a ratio of 1.38 for the all-roughage group.

The average number of services per conception was 3.11 for the grain group and 3.34 for the all-roughage group.

**P9. Carotene Losses in Freshly Cut Plant Tissues.** R. K. WAUGH, S. M. HAUGE, AND J. H. HILTON, *Purdue University, West Lafayette, Ind.*

Green plant materials with high carotene content often contain small amounts of carotene after they have been made into hay. This loss is probably due to enzymic action. Previous investigations indicate that the retention of carotene in some plant materials is greater than in others.

During the past year studies have been made to determine the losses of carotene in various types of freshly cut plant material. In these tests it was found that the destruction of carotene, due to enzymatic activity, was greater in alfalfa, red clover and sweet clover than in the oat plant, Kentucky bluegrass and brome grass. Other plants, such as corn, soybeans, and lespedeza, seem to have an intermediate enzyme activity.

**P10. Vitamin A Requirements of Dairy Cattle for Normal Growth and Reproduction.** J. H. HILTON, J. W. WILBUR, AND S. M. HAUGE, *Purdue University, West Lafayette, Ind.*

Studies have been in progress for several years to determine the vitamin A requirements of dairy cattle for normal growth and reproduction. Two groups of 60-day-old heifers were fed a vitamin-A-deficient ration until their body stores had been depleted, after which one group was fed 7,500 I.U. of vitamin A and the other group 15,000 I.U. of vitamin A daily. The source of vitamin A was a concentrate from fish-liver oil. These heifers were continued on these levels from approximately five month of age through the first gestation. Heifers of both groups grew satisfactorily.

Although these levels were adequate for growth they were inadequate for normal reproduction. Of the heifers on the 7,500-unit level, one failed to conceive and the other dropped a dead calf prematurely. Autopsy of the calf showed marked degeneration of the nervous system. Both heifers on the 15,000-unit level, likewise, dropped premature calves which were abnormal, blind and died within 48 hours after birth. These calves also showed degeneration of the nervous system, but to a less extent than the calf from the heifer on the 7,500-vitamin-A-unit level.

During the second gestation the daily vitamin A intake of two of the heifers was increased from 15,000 units to 30,000 and 45,000 units, respec-

tively. Both heifers dropped normal calves at the end of normal gestation periods.

**P11. Carotene Levels for Growth and Reproduction in Dairy Bulls. I.**  
R. JONES AND J. R. HAAG, *Oregon State College, Corvallis, Oreg.*

A group of Jersey bulls was fed whole milk to 40 days, skim milk to 90 days, and a calf meal in increasing amounts up to 3 pounds daily. Dried molasses beet pulp was fed ad libitum.

Two calves fed the basal ration only developed symptoms of vitamin-A deficiency in 6 to 8 weeks after whole-milk feeding was discontinued. The symptoms included diarrhea, eye disorders finally resulting in permanent blindness and eventually death. Blood plasma carotene values were usually of the order of 0.03 ppm.

Eight additional calves were divided into groups of 2 which respectively received alfalfa meal to supply carotene at the daily rate of 5, 20, 35, and 65 micrograms per kilogram of body weight.

Levels of 5 and 20 micrograms carotene per kilogram of body weight were not sufficient to prevent symptoms of vitamin-A deficiency, one calf dying and the other 3 becoming permanently blind. One of the latter dislocated a hip on falling and was destroyed. The carotene intakes of the other two calves, Nos. 51 and 55, were raised to 65 micrograms per kilogram of body weight at 236 and 184 days of age with the result that the bulls again began growing and the blood carotene values rose from around 0.06 to about 0.40 ppm. At 375 and 323 days of age the carotene intakes were reduced to 25 micrograms per kilogram, which has been sufficient for them to make normal growth and maintain blood carotene values of about 0.30 ppm. to their present ages of 556 and 504 days. Semen samples from the older bull No. 51 appear normal, and 3 females are probably pregnant to his service. Bull No. 55 has as yet not shown sexual desire, although other than being blind, he appears entirely normal.

The two bulls Nos. 52 and 59 fed 35 micrograms carotene per kilogram body weight have responded somewhat differently. No. 52 grew normally, had blood carotene values of 0.07 to 0.12 ppm. from 122 to 278 days of age at which time he suffered a convulsion in his pen and was found to be totally blind. No change was made in carotene intake. He has continued to grow normally and at 500 days had blood carotene value of 0.46 ppm. Semen samples appear normal. No. 59 has grown at an above normal rate, with the lowest blood carotene value 0.13 ppm. at 74 days of age and the present value about 0.50. Four females have been bred to him with two definitely pregnant after 1 and 3 services.

The two bulls Nos. 57 and 60 fed 65 micrograms carotene per kilogram of body weight have grown considerably faster than normal throughout the study without symptoms of vitamin-A deficiency. Blood carotene values

have gradually increased from about 0.2 to 0.4 ppm. as the animals have become older. Seven heifers have been bred to No. 57 with 3 probably pregnant. Four heifers have been bred to No. 60 with 2 pronounced pregnant.

**P12. Changes in Blood-Plasma Carotene Associated with Parturition and Lactation of Jersey Cows.** A. H. KUHLMAN AND W. D. GALLUP, *Oklahoma A. & M. College, Stillwater, Okla.*

Further investigation of changes in the carotene content of the blood plasma of Jerseys at parturition has provided results which are in good agreement with those previously reported by the authors. They are also in general agreement with the results obtained by Sutton and Soldner with Ayrshire, Guernsey and Holstein cows.

As in previous studies the experimental ration was composed of prairie hay, beet pulp, cottonseed hulls, cottonseed meal and minerals. Occasionally white hominy feed was included in the ration. Prairie hay in varying amounts was the source of carotene in the ration in all but a few instances when known amounts of carotene in the form of "Puratene" were given daily.

The carotene content of the hay was determined on composite daily samples at monthly intervals by Peterson's method. Plasma carotene determinations were made at monthly or more frequent intervals by colorimetric methods, the petroleum-ether soluble pigment being compared to potassium dichromate standards. Carotene determinations were made on butterfat samples of individual cows on the fifth day in lactation by the spectrophotometric procedure of Fraps.

Data which were secured from 63 calving records of 30 high-grade Jerseys showed that at parturition there was a decrease in plasma carotene in 52 cases, an increase in 9 cases and no change in 2 cases. The average plasma carotene values for the entire group were 169 micrograms per 100 ml. plasma before calving and 134 micrograms after calving. This is an average decrease of 21 per cent. These results when grouped according to the carotene intake of the individual cows before and after calving either as milligrams per day or as micrograms daily per pound body weight, showed the general decline in plasma carotene previous to and directly after calving for all groups. The percentage decrease in plasma carotene was directly related to the amount present previous to parturition; it was less closely related to carotene intake since some cows maintained relatively high plasma carotene values despite low intakes. For cows on low levels of carotene intake and having correspondingly low plasma carotene values, 70-72 micrograms per 100 ml. of plasma, the decrease in plasma carotene at calving was less than 2 per cent. When plasma carotene values were high, over 200 micrograms per 100 ml. of plasma, the decrease at calving time was over 30 per cent. Groups between these extremes showed intermediate values. At

present there seems to be no explanation for these changes in plasma carotene. They may be associated with milk secretion, absorption or storage, or with a more efficient conversion of carotene to vitamin A during a critical period.

There is apparently a rather definite relationship between the carotene content of the blood plasma after calving and the carotene content of the butterfat. Carotene values of the butterfat of milk produced on the fifth day in lactation were: 0.88, 1.51, 2.90 and 3.32 micrograms per gram of fat when the blood-plasma carotene values after calving were, respectively: 67, 114, 174 and 262 micrograms per ml. of plasma.

**P13. Vitamin E in the Nutrition of Cattle.** T. W. GULLICKSON, L. S. PALMER, W. L. BOYD, AND F. C. OLSON, *Minnesota Agricultural Experiment Station, St. Paul, Minn.*

This is a progress report and relates to a study started in 1938, the object being to determine the importance of vitamin E in the nutrition of cattle, especially in relation to reproduction. In 1940 we reported in regard to the vitamin-E potency of a considerable number of feedstuffs and also the methods of assaying used. The present report deals with the results obtained with cattle fed rations made up entirely of feeds devoid of vitamin E as determined by both bioassay and chemical tests.

A total of 22 animals, 15 females and 7 males, of various breeds have been used to date (including one female and one male fed alpha tocopherol as a supplement) for more or less extended periods of time. Records have been kept of growth, amount of feed fed, physical and sexual development and general well-being of each animal. Determinations have also been made at monthly intervals of the calcium and inorganic phosphorus and the ascorbic acid content of the blood plasma.

Growth and physical and sexual development in all cases have been normal. Of the eleven females of breeding age, including the check animal, ten have calved normally. The other heifer died suddenly from unknown causes about six weeks before she was due to calve; however, the fetus present was normal. All calves have been normal in size and very vigorous at birth. In only two of the eleven heifers represented was more than one service required for conception. The sire used in each case was raised on a vitamin-E-free ration. A considerable number of heifers were discontinued after calving and therefore little information is available in regard to second calvings.

The ascorbic acid content of the blood plasma of cattle on vitamin-E-free diets was similar to that of the check animals and those fed normal rations. Some of the vitamin E ingested by cattle fed normal rations was voided in their feces. None was found in the feces of those fed the vitamin-E-free rations. No detectable amount of vitamin E has been found in the tissues (liver, muscle, body fat, testes) of cattle fed the deficient rations.

P13a. The Value of Fat in Alfalfa Hay Rations. J. H. BYERS, V. R. SMITH, AND I. R. JONES, *Oregon State College, Corvallis, Oreg.*

Cows fed alfalfa hay supplemented with salt and disodium phosphate have produced in our herd only about 70 per cent as much fat-corrected milk as expected from the amount of total digestible nutrients consumed.

Experiments with six cows were conducted over a period of two years, during which various concentrates, including ground soybeans, skimmilk powder, sugar, fish meal, meat meal, blood meal, molasses beet pulp, dehydrated molasses, wheat bran, soybean meal, peanut meal, oat groats, and wheat middlings were fed over 4-week periods in comparison with periods of feeding on alfalfa hay supplemented only with salt and disodium phosphate. The decline in production when the cows received a ration of alfalfa hay alone, whether in early, middle, or late lactation, was practically always much more rapid than when part of the alfalfa hay was replaced by calculated equal amounts of total digestible nutrients in the various concentrates used. The production was particularly higher during the periods when soybeans were fed. Skimmilk powder seemed to be the next most efficient of the concentrates used.

The results with these various concentrates might indicate that the digestible nutrient system overrates the productive value of alfalfa hay when it constitutes the principal or sole source of nutrients other than minerals.

In order to check further the relationship between the fat content of the ration and the production of milk and butterfat, four additional cows were carried through complete lactations on rations of alfalfa hay and ground soybeans, alternated at 6-week intervals with alfalfa hay and soybean meal. The average fat content of the dry matter of the ration was 5.12 per cent when the ground soybeans were fed and 2.55 per cent when soybean meal was used.

In this experiment, twenty-five feeding periods allowed seventeen comparisons to be made of the high- and low-fat rations. The high-fat ration favored milk production in five; fat percentage in fourteen, the average increase being 12.8 per cent; pounds of butterfat in fifteen, the average increase being 11.0 per cent; and pounds of fat-corrected milk in twelve, the average increase being 7 per cent.

The feeding of ground soybeans or soybean meal with alfalfa hay allowed milk and butterfat production to be maintained above or at least equal to what is expected of cows on good rations throughout the lactation.

P14. Studies on Ketosis in Cattle. J. C. SHAW AND L. D. MATTERSON, *Departments of Dairy Industry and Poultry Husbandry, Storrs Agricultural Experiment Station, Storrs, Conn.*

Nine cows with severe ketosis as shown by ketonemia, hypoglycemia and lack of appetite had an average blood-plasma carotene level of 321.8 micro-

grams per 100 ml. of plasma (range 131 to 768). Vitamin A was determined on seven of these animals and was found to average 24 micrograms per 100 ml. of plasma (range 14.4 to 33.6). The oral administration of from one to four million units of vitamin A per day for as long as three weeks was completely ineffective in improving the appetite or alleviating the hypoglycemia and ketonemia. Turning the cows out on pasture usually resulted in rapid recovery, in some cases in as little as two to three days.

**P15. Symptoms of Scurvy Observed in a Herd of Cattle.** C. W. DUNCAN, C. F. HUFFMAN, R. MITCHELL, JR., AND J. T. REID, *Michigan State College, East Lansing, Mich.*

Preliminary field observations made during the past winter and spring indicate that cattle may develop symptoms of scurvy under some conditions of livestock management. More than 35 cows and calves on one farm have died as a result of the disease.

The symptoms of the disease in the surviving animals were characterized by loss of weight and fatigue. Mouth examinations indicated that the interdental papilla, the marginal or the alveolar gingiva may be involved to a greater or lesser degree. The marginal gums in some of the animals were bluish to reddish in color, frequently swollen but in some cases receding from the teeth. Eroded areas were most frequently found on the upper dental pad, to a lesser extent on both the upper and lower pads and to a minor degree on the lower pad. There was no excessive salivation but less than normal seemed to be the rule. Lesions on the nose and lips were commonly found. The skin of the animals manifest a rough, dry scaly condition and some small reddened areas due to subcutaneous hemorrhage were observed.

The blood picture of these animals was characterized by low plasma ascorbic acid values, low hemoglobin values and low magnesium values. The addition of chlorobutanol to the regular ration caused a marked increase in the plasma ascorbic acid values, gradual healing of the eroded areas on the dental pads and a marked improvement in the skin condition.

**P16. The Effect of the Level of Negative Pressure on the Rate of Milking.** VEARL R. SMITH AND W. E. PETERSEN, *University of Minnesota, St. Paul, Minn.*

Rates of milk extraction by varying negative pressure were determined on eight cows. The rates were ascertained by suspending the milking machine pail from a scale and taking readings every ten seconds. Letdown was stimulated by a 15-second wash and massage two minutes before the teat cups were put on. Comparisons were made of rates of flow at 10, 12, 14, and 16 inches of mercury negative pressure on the line. The rate of pulsa-



tions was 50 per minute for all observations. Scale readings were made on two successive milkings for each negative pressure level and each level was repeated until there were ten milkings for the four levels.

The rate of flow at each negative pressure level varied markedly with individual cows. At ten inches of negative pressure the average rate of milk flow per ten seconds ranged from 0.43 to 0.58 pounds, at twelve inches from 0.50 to 0.68 pounds, at fourteen inches from 0.56 to 0.75 pounds, at sixteen inches from 0.66 to 0.84 pounds for the eight cows included in the study. The results show increased rates of flow with increases in negative pressure within the limits studied. There is not a straight line increase in rate of extraction with each increase in negative pressure for all cows. This phenomenon is based on two functions of negative pressure; first, increased opening of the meatus which varies with different cows; second, the increased rates of flow with increases in negative pressure.

These data when treated statistically show that the differences in rates between the different levels of negative pressure are highly significant.

#### P17. Studies of Mammary Gland Carbohydrate Metabolism in Vitro.

C. B. KNOTT AND W. E. PETERSEN, *University of Minnesota, St. Paul, Minn.*

A series of experiments have been conducted in the study of the carbohydrate metabolism of the mammary glands involving incubation of tissue slices and the perfusion of excised mammary glands. It has been possible to increase the concentration of tissue glycogen in excised mammary glands by perfusion with high concentrations of blood glucose. It appears that glycogen can be converted in part to lactose during incubation of the previously perfused tissue. In perfusions where the concentration of blood glucose was greatly increased up to 5,611 mg. per cent there was not a significant change in the lactose concentration of the milk secreted during 6 hours of perfusion.

Lactose was formed during incubation of tissue slices with glucose, glucose and lactic acid, maltose, and glycogen. Lactic acid was formed by incubating tissue alone as well as from added glucose, maltose, glycogen, pyruvic acid, and citric acid. Citric acid was formed from glucose, lactic acid, pyruvic acid, maltose, and glycogen. Incubating mammary gland tissue slices utilize significant amounts of pyruvic acid.

Introduction of insulin greatly decreased lactose formation by the perfused mammary glands even though a hyperglycemia was maintained during the perfusions.

$\beta$ -hydroxybutyric acid is utilized by mammary gland tissue slices. The rate of utilization bears a definite relation to the concentration present. Citric acid is not formed in an appreciable amount from  $\beta$ -hydroxybutyric acid during incubation.

P18. Pre-Partum Milking. E. A. KEYES, J. J. REID, S. I. BECHDEL, A. A. BORLAND, A. L. BEAM, AND P. S. WILLIAMS, *Pennsylvania Agricultural Experiment Station, State College, Pa.*

Pre-partum milking, or pre-milking as it is more commonly called, involves the milking of cows and heifers before freshening.

The work at The Pennsylvania State College has been done with special note being taken as to the effect on the cow and the bacteriological picture of the milk. Fifty-five cows have been on experiment so far. Twenty-five have been pre-partum milked 2 to 16 days and the remaining 30 have been used as checks. Quarter samples are taken on all pre-partum milked cows each milking before freshening and at least 4 days after freshening. Quarter samples are taken on all check cows at least 4 days after freshening. Some trends and results are indicated by the following figures. Thirty-one and two-tenths pounds milk per day is the highest on any pre-partum-milked cow before freshening to date.

	<i>Pre-milked cows</i>	<i>Not pre-milked</i>
Range, lbs. milk/milking before freshening	0.3 to 20.0	
“ “ “ “ after “ (4 days)	1.0 to 37.5	2.0 to 35.5
Range, % fat in milk before freshening	0.2 to 11.0	
“ % “ “ “ after “ (4 days)	1.9 to 9.4	0.9 to 10.5
Range, % total solids in milk before freshening	9.46 to 39.05	
“ % “ “ “ after “ (4 days)	10.47 to 25.19	9.92 to 34.75

The per cent total solids show a great deal of variation until about the seventh day before freshening when they become more uniform and gradually approach normal at time of freshening. Three days after freshening, the pre-partum-milked cows and the non-pre-milked cows average the same in per cent total solids.

The per cent butterfat stays between 4 and 5 per cent from the eleventh day before calving. As calving approaches, the per cent fat increases to about 5 per cent and stays around 5 per cent for at least 4 days after freshening. The non-pre-partum-milked cows average about 4.5 per cent at day of freshening and stay just under 5 per cent for at least 4 days after freshening.

The average total pounds of milk for the pre-partum-milked cows starts at around a pound a day and increases at the rate of 1 to 2 pounds a day until the third day before calving, when it increases at the rate of 5 to 6 pounds a day. A total of 25 pounds was reached the day of calving. This increases to 42 pounds the fourth day after calving. The non-pre-partum-milked cows start at 21 pounds the day of freshening and increase to 37 pounds the fourth day after freshening.

The carotene in the blood was determined every two days beginning with the sixth day before freshening and continuing to the fourth day after freshening. The blood contained 0.31 mg./100 cc. the sixth day before freshening

and suddenly dropped to 0.1 mg./100 cc. the day before freshening. After freshening there was a rise to 0.25 mg./100 cc. when it again dropped to a low of 0.03 mg./100 cc. The milk carotene gave the opposite picture. Starting at 0.03 mg./100 cc. and rising to 0.24 mg./100 cc the day before freshening. The day of freshening it increased to 0.4 mg./100 cc. then decreased, reaching 0.1 mg./100 cc. the fourth day after freshening.

The bacteriological picture is very interesting. Streptococcus of some form has been isolated from every cow whether she has been pre-partum milked or not. Alpha and beta streptococcus have appeared most frequently, with a few gamma. Excessive numbers of leucocytes have been found in all cows. The concentration of streptococci and leucocytes has been highest in the first milkings. The streptococci gradually disappear until 4 days after freshening when they cannot be detected by the regular bacteriological tests in most of the animals. During this period the number of leucocytes dropped to normal. By special tests with the use of a centrifuge the streptococcus can be detected in all cows at any time, which indicates they are normal flora of the cow's udder. When a cow has indurations in the udder or has been injured or ill, we have found a greater concentration of streptococci in the udder than with cows that appear perfectly normal in all respects.

Four calves from dams which had been pre-partum milked became ill. They had scours and showed signs of general inactivity. Five cc. of "Carotone" (a carotene preparation) administered daily has brought them back to normal in 7 days.

This experiment will be concluded next December. A more detailed report and conclusions will be drawn at that time.

**P19. The Use of Simplified Diets in the Study of the Fat Metabolism of the Mammary Gland.** O. W. KAUFMANN AND J. C. SHAW, *Department of Dairy Husbandry, Storrs Agricultural Experiment Station, Storrs, Conn.*

The effect of certain simplified diets upon the iodine number and Reichert-Meissl value of milk fat was studied in an attempt to obtain additional evidence as to the precursors of the lower fatty acids of milk fat. Diets consisting solely of skim milk, and corn starch and glucose resulted in an increased Reichert-Meissl value and a decreased iodine number within 48 hours. To determine whether the synthesis of the lower fatty acids from carbohydrate was direct or indirect, sufficient insulin was injected into fasting cows to maintain a hypoglycemia for approximately 30 hours. The rate of decline of the Reichert-Meissl value did not differ significantly from that observed on fasting alone. It is concluded that the decrease of the lower fatty acids on fasting is not due to a decrease in the blood-glucose level.

**P20. Effect of Feeding Cod-Liver Oil by Two Different Methods on Butterfat Test.\*** L. A. MOORE, G. T. HOFFMAN, AND M. H. BERRY, *Maryland Agricultural Experiment Station, College Park, Md.*

It has been known for some time that the feeding of cod-liver oil to cows causes a depression in butterfat test. This reduction in test is thought to be due to the unsaturated fatty acids of the C<sub>20</sub> and C<sub>22</sub> series. It has also been noted that other highly unsaturated oils such as soybean oil and corn oil may cause some depression in test although the results are at variance. When corn oil or soybean oil are fed as corn grain or soybeans, there is no depression and sometimes an increase in test.

These differences suggested the possibility that the feeding of an oil, as such, so that a large amount of oil would be absorbed within a short period of time, was different than where the oil was fed in its original state in the seed so that only small portions of oil would be absorbed over a longer period of time. This difference might be due to the amount of saturation of the unsaturated fats by the animal during digestion and absorption which would have some effect on butterfat test.

Consequently, cod-liver oil was fed to two groups of cows by two different methods. One group was fed 5 to 7 oz. of cod-liver oil in one dose each day for 3 to 5 days. The other group was fed the same amount of oil but divided into 12 feedings for each day.

The results showed that when the oil was fed in one feeding, a marked depression of test occurred as has previously been reported. However, when the same amount of oil was distributed into 12 separate feedings over the 24-hour period, generally no such marked depression of test occurred. The iodine number was markedly elevated when the oil was fed as one feeding. When it was fed in 12 separate feedings, the iodine number was elevated but not as markedly as in the previous case.

These results with cod-liver oil, therefore, indicate that during the digestion and absorption of unsaturated fats there is some saturation taking place. The amount of saturation which takes place depends on the quantity and rapidity with which the oil is absorbed which in turn affects the butterfat test. If this same principle applies to other oils, it might explain the variation of results obtained on butterfat test by other investigators.

**P21. Further Observations on the Initiation and Maintenance of Lactation in Dairy Cattle.\*** RALPH P. REECE, *New Jersey Agricultural Experiment Station, New Brunswick, N. J.*

Additional attempts have been made to induce and maintain lactation in dairy cattle by the administration of hormones.

\* Scientific Paper No. A86<sub>a</sub>, Contribution No. 1944 of the Maryland Agricultural Experiment Station (Department of Dairy Husbandry).

\* Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Husbandry.

A 33-month-old barren Jersey heifer, 381X, received a total of 273 mg. of diethylstilbestrol dipropionate over a 14-week period. She produced 8,046 lb. of milk and 383 lb. of butterfat in 305 days. At the beginning of the 11th month of lactation 381X was producing 16 lb. of milk per day. At this time she received four weekly injections of 2 mg. diethylstilbestrol dipropionate plus 5 mg. testosterone propionate. Milk production eventually declined to 11 lb. per day and then increased to 19 lb. per day. In the following six months 381X produced 2,716 lb. of milk.

A 3-year-old Jersey heifer, 384X, received a total of 273 mg. of diethylstilbestrol dipropionate in 14 weeks. Milking was begun 14 days after the last injection and continued for five months. The level of milk production gradually increased, reached a peak daily production of 16.5 lb., and then declined to 12 lb. per day in the fifth month of lactation.

An Ayrshire cow, 472, that had calved five times was open and dry (Apr. 5). She was injected with 5 mg. of diethylstilbestrol dipropionate subcutaneously twice weekly for six weeks. Ten mg. and then 20 mg. of diethylstilbestrol were injected subcutaneously twice weekly for one week. At no time did the udder fill with secretion. Fifteen mg. of diethylstilbestrol were injected subcutaneously on July 9, 10, 11, 15, 19, 23, 26, 30, and Aug. 3 and 7. Milking was started July 20 and the animal reached a peak daily production of 17.8 lb. on Aug. 25.

A 26-month-old Jersey heifer was open and she was injected with 5 mg. of diethylstilbestrol dipropionate in conjunction with 5 mg. of testosterone propionate twice weekly for eight weeks. The heifer's udder "made-up" at the beginning of the fifth week of the injection period and as a result daily milking was begun. Following 10 days of once daily milking the heifer was put on a twice-daily milking schedule. She attained a peak daily production of 14.8 lb. in the second month of lactation.

A 4-year-old Jersey heifer that had been through one normal lactation was dry and open (July 21). She received 5 mg. of diethylstilbestrol dipropionate subcutaneously twice weekly for eight weeks. Ten mg. of diethylstilbestrol were injected subcutaneously on Sept. 15, 16, 17, 22, 25, 28, and Oct. 1, 4, 6, and 8. Even though daily milking was carried out this animal never came into milk production.

## P22. The Response of Louisiana Milk Cows to Iodinated-Casein Feeding.

D. M. SEATH, CECIL BRANTON, AND A. H. GROTH, *Louisiana Agricultural Experiment Station, Baton Rouge, La.*

Two trials have been completed on the feeding of iodinated casein (synthetic thyroprotein) to lactating Louisiana dairy cows. In the first trial two groups of 3 cows each were used under the single reversal design. Group I received iodinated casein for a period of 8 weeks and group II for 7 weeks. A 1-week preliminary, and a 3-week transition period between tests, were

sed. Cows were fed 15 grams of the iodinated casein daily. In the second trial two groups of 6 cows each were used, one group being fed iodinated casein for an 11-week period.

In the first trial greater yields of milk with a higher percentage of butterfat were secured while the cows were being fed iodinated casein. These increases were accompanied by losses in body weight with certain individual cows showing heavy losses, while others were less affected. The iodinated casein also caused a substantial increase in pulse rate and a slight increase in body temperature. Cows used in this trial had been fresh an average of less than three months prior to the first experimental period.

The second (continuous feeding) trial used cows that had been fresh an average of  $5\frac{1}{2}$  months. For the first 6 weeks responses in milk production due to the iodinated-casein feeding were similar to those in the first trial, following which little differences were shown between the test and control groups. Again, there was a great stimulation to pulse rate and a slight increase in body temperature. Body-weight losses were again noted. An attempt was made to prevent excessive losses of weight by periodically varying the amount of iodinated casein fed from the original rate of 15 grams per 1,000 pounds live weight, but only moderate success was achieved.

In each experiment atmospheric temperatures (as measured by dry and wet bulbs) had an apparent marked influence on the body temperatures of both test and control cows. This influence was much more marked than was the influence of the iodinated casein. For example, in the first trial body temperatures of the group on iodinated casein averaged  $105^{\circ}$  F. at 5 P.M. on two days when atmospheric temperatures averaged  $93.5^{\circ}$  F. Likewise, cows not receiving iodinated casein in the second trial averaged  $104.6^{\circ}$  F. during a period of 2 days when atmospheric temperatures averaged  $96.5^{\circ}$  F. These high body temperatures raise the question relative to the advisability of feeding compounds such as iodinated casein to Louisiana milk cows during the warmer months of the year.

**P23. Studies on the Utilization of Thyroprotein by Ruminants.\*** C. W. TURNER AND E. P. REINEKE, *Department of Dairy Husbandry, University of Missouri, Columbia, Mo.*

By the iodination of casein and other proteins rich in the amino acid tyrosine (under carefully controlled conditions), it has been found possible to produce an intramolecular synthesis of thyroxine, the hormone of the thyroid gland. This synthetic thyroprotein has been shown to increase the milk yield and fat percentage when fed to dairy cattle at the rate of 1.5 to 2.0 gm. per 100 lb. body weight. In comparison with the oral requirements of non-ruminant animals, it appeared that thyroprotein was being utilized inefficiently by cattle and other ruminant animals.

\* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 949.

As the organisms of the rumen are believed to break down food protein and synthesize organismal protein, it seemed possible that the losses of thyroprotein might be due to the partial breakdown of this protein and its recombination in the organisms in an inactive form.

In order to study this problem, sheep were selected as assay animals, and the loss in average body weight following the administration of the thyroprotein for a period of 2 weeks was used as an indication of response.

The results to date indicate that about 5 per cent as much thyroprotein is required when administered subcutaneously as when given orally by capsule. When thyroprotein is suspended in water and administered as a drench, twice the oral dose given by capsule is required. A series of substances such as stearic acid, paraffin, beeswax, rosin, clarite and vinylite have been applied to the particle in order to "protect" the thyroprotein in the rumen. Every coating tried so far has reduced the physiological potency rather than increased it.

In order to determine whether the losses occur in the rumen, a permanent cannula has been inserted into the abomasum or true stomach of a group of sheep. By administering the thyroprotein into the abomasum the entire rumen system can be by-passed. However, no difference was noted in the effectiveness of thyroprotein given by mouth or directly into the true stomach. From this it is concluded that the apparently low rate of absorption of thyroprotein in the digestive tract is not due to inactivation in the rumen.

Thyroprotein has been hydrolyzed with acid to determine whether better utilization would be obtained with a predigested product. Preliminary trials show that the hydrolysate is about twice as effective as the original thyroprotein when given either by mouth or directly into the abomasum. Thus the poor utilization of thyroprotein by ruminants may be explained in part by its low digestibility in the ruminant alimentary system.

**P24. Studies of Thyroid Physiology by Use of Thiourea and Its Derivatives\*** E. P. REINEKE, A. B. SCHULTZE, AND C. W. TURNER, *University of Missouri, Columbia, Mo.*

It has been discovered recently that formation of thyroxine by the thyroid gland can be inhibited by the administration of thiourea, thiouracil and other thiourea derivatives. This results in a compensatory hypertrophy and hyperplasia of the thyroid gland due to increased output of thyrotropic hormone by the anterior pituitary in response to the induced thyroid deficiency. By the administration of thyroxine, thyroid gland substance, or artificial thyroprotein, the pituitary thyrotropic hormone can be held in check, and the size of the thyroid of thiourea- or thiouracil-treated animals is reduced.

\* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 951.

a proportion to the dosage of thyroïdal substance. It is thus possible to study, without surgical intervention, the effect of either thyroid deficiency or thyroid excess on physiological processes such as growth, lactation and reproduction. Also, the actual thyroid hormone output can be measured by determining the amount of thyroxine required to return the thyroid glands of thiouracil-treated animals to the normal weight. Thus the effect of such factors as age, breed, species, sex, diet and environment on thyroid hormone output can be studied.

Data are presented to show the effect of thiouracil alone and thiouracil plus graded amounts of thyroxine on the carbon dioxide output and thyroid gland weights of male albino rats. In rats receiving 0.1 per cent thiouracil in their drinking water the carbon dioxide output had declined 22 per cent and the thyroid gland weight per 100 gms. body weight had increased 117 per cent at the end of two weeks. When d,l-thyroxine was injected the carbon dioxide output increased and the thyroid weight decreased in proportion to dosage, both reaching normal levels within the range of 3.5 to 4.8 micrograms of d,l-thyroxine daily per rat.

Preliminary experiments were conducted on lambs and kids to determine the amount of thiourea required to suppress their growth and thyroid secretion. Varying degrees of growth suppression and cretinism were produced in lambs by the injection of 360 to 540 mg. of thiourea daily. The same dosage was effective when given orally. Goat kids were given thiourea starting at approximately one month of age, continued on treatment for 30 days and then slaughtered for examination of their glands and general condition. Maximum thyroid gland enlargement was obtained on a thiourea dosage of 360 mg. daily. Inasmuch as many drugs are known to be utilized poorly in the ruminant digestive system, it is of particular interest that thiourea was fully as effective when given orally as by the subcutaneous route.

Since thiourea and its derivatives will block out thyroid activity, and it had been established previously that an active thyroid is essential for maximum milk production, it was expected that thiourea would cause a decrease of production in lactating animals. Given orally to seven lactating goats, 750 mg. of thiourea daily for two weeks caused a 25 per cent decline in milk yield. Milk production began to recover by the seventh day after the dosage was stopped and closely approached the initial yield by the end of the third week. The application of this new technique to measurement of normal thyroid hormone output in dairy cows is discussed.

**P25. The Effect of Pitocin on Milk Lipase.** PHILIP L. KELLY, *University of Arkansas, Fayetteville, Ark.*

The method of lipase analysis used in these studies has been described. In the work reported here, the hormone, pitocin, was added to series of



samples of dried milk to study its effect on the milk lipase in comparison with similar samples to which no hormone was added.

With tributyrin as the substrate eleven series were carried out of which three were from normal milk, seven were from milk which developed rancidity and one was of unknown quality. In all but two of these series the hormone definitely increased the tributyrinase activity sometimes by almost 100 per cent. The two exceptions were both produced by the same cow and would have produced intense rancidity. In both, the addition of pitocin decreased lipase activity.

With triacetin as the substrate, in two series of normal milk the addition of pitocin increased lipolytic hydrolysis. This was also true for one series of normal milk samples with tripalmitin as the substrate.

The three series with ethyl-oleate including 119 individual analyses showed different results. In one series in which an unknown quality of milk was used, lipase activity was decreased to 10 per cent of its former activity. In the two series with normal milk, one showed no differences and in the other the lipase activity was increased.

When butter oil was the substrate the results again varied with four series showing increases, three showing decreases and one showing no definite difference. The effect of the hormone did not appear to be related to the type of milk used. In one of the series showing a decrease, samples of the same batch of dried milk showed a definite increase with tributyrin as the substrate. The remaining two series which showed decreases were from the same milk as those series which showed decreases in tributyrinase activity.

In four series either whole or dried milk samples were used with butter oil as the substrate. Distillation analyses were compared with the total fat analysis to learn whether the hormone had a selective effect on the short chain triglycerides. This was found to be true with the volatile fatty acids accounting for a large proportion of the changes produced. In one series with dried milk the total hydrolysis of the butter oil was not changed, but there was an increase in the volatile fatty acids indicating the hormone had increased the hydrolysis of some of the triglycerides and apparently inhibited the hydrolysis of others.

**P26. Factors Affecting the Chylomicron Count.** DWIGHT ESPE AND C. Y. CANNON, *Iowa State College, Ames, Ia.*

The number of chylomicrons in the blood of calves on grain and hay follow an entirely different cycle from that exhibited by young calves fed principally milk. The chylomicron count is much higher when calves are fed whole milk than when skim milk plus an equivalent amount of fat is given.

The number of chylomicrons can be reduced by feeding a watery extract of pancreas high in lipase.

- P27. **Morphology of the Teat in Relation to Milking and Trauma.** W. E. PETERSEN, C. B. KNOTT, AND W. L. BOYD, *Divisions of Dairy Husbandry and Veterinary Medicine, University of Minnesota, St. Paul, Minn.*

Four hundred teats including the gland sinus were obtained from dairy cattle, were dissected for gross structures, and 25 representative specimens were sectioned and studied microscopically. Great variations were noted in structures, either normal or due to pathology, that would affect milking by machine.

The annular fold demarking the gland and teat sinuses varied greatly in location and extent. In several cases there were two or three folds. Frequently this fold was sufficiently below the floor of the udder to be compressed by the teat cups so as to completely occlude the passage of milk from the gland into the teat. In many cases the fold was above the udder floor where compression by the teat cup is not possible.

Only about 10 per cent of the specimens studied were found to be free of pathology that could be detected grossly upon dissection. The pathologic findings varied from small inflammatory and fibrotic areas that could not be palpated to extensive fibrotic growths that practically occluded the teat sinus. In most cases this pathology was located in the upper part of the teat sinus and presumably had its origin from trauma produced by milking. In several cases pathology in the lower teat sinus could be attributed to previous severe trauma to that portion of the teat as evidenced by the finding of external scar tissue.

Histological examination revealed accessory secretory glands in the upper walls of the teat sinus in nearly all of the specimens and always in the gland sinus. These glands varied greatly in size, location in the teat and depth in the teat wall. Varying stages of pathology were observed in these glands. It is suggested that because of their structure and location they are frequently traumatized by milking which fact is a prominent predisposing factor in mastitis.

- P28. **Threshed Peanut Hay as a Roughage for Dairy Cows.** A. H. KUHLMAN AND H. W. CAVE, *Oklahoma A. and M. College, Stillwater, Okla.*

The growing of peanuts as an essential war crop in many sections of the cotton belt has made available large amounts of cured vines commonly known as threshed peanut hay. If carefully conserved this by-product of the peanut crop can be used satisfactorily as a substitute for other legume roughages.

Two ninety-day experiments conducted according to the double reversal plan have been completed comparing the value of threshed peanut hay and alfalfa hay as roughages for dairy cows. In each trial a very good grade of

peanut hay, which was of good green color and very leafy, was compared with alfalfa hay of corresponding quality.

In the first trial in which threshed peanut hay produced in 1942 was used the daily hay allowance of each cow was slightly less than 1.6 pounds for each 100 pounds of body weight. Alfalfa hay fed at this level supplies an amount of total digestible nutrients equivalent to the requirements for body maintenance. The concentrate mixture fed in this trial consisted of 400 pounds ground yellow corn, 200 pounds ground oats, 200 pounds wheat bran, 100 pounds cottonseed meal (43 per cent protein), and 9 pounds of salt.

Nine cows when fed the alfalfa hay ration on the average produced 16.44 pounds of milk daily testing 4.25 per cent fat, equivalent to 17.06 pounds of 4 per cent fat-corrected milk. For each 100 pounds of 4 per cent milk produced they consumed 80.67 pounds of alfalfa hay and 62.03 pounds of concentrates. When fed the peanut hay ration these cows on the average produced 16.08 pounds of milk testing 4.36 per cent, equivalent to 16.96 pounds of 4 per cent milk. For the production of each 100 pounds of 4 per cent milk, 80.65 pounds of peanut hay and 61.03 pounds of concentrates were consumed.

In the second trial in which hay grown in 1943 was used, both kinds of hay were fed at the rate of 1.8 pounds of hay for each 100 pounds of body weight. The concentrate mixture used consisted of 400 pounds ground yellow corn, 200 pounds ground oats, 200 pounds wheat bran and 8 pounds of salt. Twelve cows when fed the alfalfa hay ration on the average produced 31.86 pounds of milk daily testing 3.64 per cent fat, equivalent to 30.13 pounds of 4 per cent milk. On the peanut hay ration these cows on the average produced 32.10 pounds of milk daily testing 3.68 per cent fat, equivalent to 30.55 pounds of 4 per cent milk.

To produce 100 pounds of 4 per cent milk the cows on the check ration consumed 61.42 pounds of alfalfa hay and 47.91 pounds of concentrates, as compared with 61.04 pounds of threshed peanut hay and 46.36 pounds of concentrates on the experimental ration.

The results of this study show that threshed peanut hay of good quality is equivalent in feeding value to alfalfa hay of similar quality.

**P29. The Use of Urea in Commercial Dairy Feeds.** W. H. HASTINGS,  
*Lindsey-Robinson & Co., Inc.*

Urease activity in ingredients and silage was tested. Some ammonia was liberated after two days when urea was mixed with soybean silage. Urease activity, probably of microbiological origin, was noted in all silage samples after seven days at ordinary temperatures.

Palatability of ingredients containing 50 to 60 lb. urea per ton was tried with Holsteins, Guernseys and Jerseys. No case of feed refusal, digestive disorder or "off-feed" could be traced to the presence of urea.

Feeding tests were made on a herd of registered Holsteins. Last year, the third consecutive year on official test, the herd average was 437 pounds of butterfat and 12,933 pounds of milk. Seven cows were chosen for the test group. These were average in age, previous production, and had freshened between September and November of last year. The rest of the herd received the control ration, which the whole herd had been getting for several years. Fifteen cows with an average history of production, freshened between September and November of last year, were chosen for record comparisons as the control group. Twice a day the cows were milked and the milk weighed. Official fat tests were used. All silage, hay and grain concentrates were weighed at each feeding.

Urea was added to the grain concentrate in such a proportion that in November, 17 per cent of the total protein from hay, silage, and concentrate was non-protein nitrogen. In December, 21 per cent of the total protein came from non-protein nitrogen. In January, 25 per cent and in February, 29 per cent of the total nitrogen was from urea. The February ration was continued throughout the rest of the test.

Milk production, butterfat, milk protein and body weight for cows on the test group did not vary significantly from the herd average from November, 1943, to June, 1944. If urea were not used as a source of protein, the cows on the test group were getting 0.5 lb. of protein per day less than they required for maintenance and milk production.

Another group of seven cows was selected for studying the effect on milk production and palatability of changing feeds suddenly. Abrupt changes of feeds with and without urea has no effect on milk production or palatability of feed.

### **P30. Corn Silage Made with the Addition of Urea and Its Feeding Value.**

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Corn silage, made with the addition of 10 pounds of urea per ton of corn ensiled was fed along with low-protein concentrates and hay to a group of cows in a single reversal experiment for 100 days. Another group was fed similarly except that the urea was mixed with the concentrates instead of being ensiled with the corn. Both groups maintained their production of milk exceptionally well and the method of feeding urea had no significant effect. The cost of urea for these cows, which were producing 4 gallons of milk daily was a trifle over 1 cent per cow per day.

Although the seepage of juice from the silage amounted to 3 per cent there was no excessive loss of urea and there was no greater concentration of urea in the lower part of the silo. The addition of urea did not reduce the losses of dry matter, nitrogen, and carotene in the silo. Moderate addi-

tions of urea either to the silage or to the concentrates slightly impaired the palatability; heavier additions impaired it still more.

Ten of the cows were continued on experiment for 40 days to compare soybean meal with urea in a grain ration, 100 pounds of soybean meal being used to replace 14 pounds of urea and 100 pounds of low-protein concentrates. The decline in milk for the 40 days was 3.2 per cent as against 3.0 per cent for the preceding 40 days when urea was fed.

Small silos were filled with corn and heavier additions of urea. A Holstein cow ate in 61 days an average of 104 pounds of silage a day containing 1.17 pounds of urea. A Jersey cow ate in 60 days an average of 58 pounds containing 0.58 pound of urea. Post-mortem examination revealed no evidence of harm to either cow.

**P31. Urea in Sorghum Silage.\*** GEO. K. DAVIS, R. B. BECKER, P. T. DIX ARNOLD, C. L. COMAR, AND SIDNEY P. MARSHALL, *Florida Agricultural Experiment Station, Gainesville, Fla.*

Sweet sorghum from an experimental planting was ensiled in 4 pilot silos, each holding about a ton of silage. Urea, in water solution, was mixed with the sorghum at the rates of 0, 10, 30 and 50 pounds of urea per ton in the different silos. Temperature changes in two of the silos, those containing 0 and 30 pounds of urea, were followed by means of thermocouples. The temperature increases were not large and those in the urea silage were slightly higher than those of the control. Some temperature differences were noted in the silos after opening. Temperature change did not appear to be related to urea content of the silage. Crude protein determinations demonstrated some migration of the nitrogen in the silos but the loss was slight. Urea determinations on silage as it entered the silos and after removal indicated that comparatively little of the urea was changed. Free ammonia was noticed in the silage containing the highest level of urea but in none of the other silages. Determination of pH in the silages gave values between 3.5 for the control and 7.6 for the 50-pound level of urea.

Palatability observations were made on the sorghum silages with dairy cows. The cattle ate the silages containing no urea and 10 pounds of urea per ton about equally well followed by that containing 30 pounds per ton. The 50-pound-per-ton level of urea caused this silage to be refused entirely until much of the free ammonia had disappeared from it.

**P32. Urea-Treated Corn Silage vs. Untreated Corn Silage as a Feed for Lactating Dairy Cows.** G. H. WISE, J. H. MITCHELL, J. P. LAMASTER, AND D. B. RODERICK, *Clemson Agricultural College, Clemson College, S. C.*

This is a report of a preliminary study designed to determine the feeding value of corn silage treated with urea in aqueous solution, two pounds of

\* Published with the approval of the Director of the Florida Agricultural Experiment Station.

crystals (46 per cent nitrogen) per gallon of solution. This was added at the cutter at the rate of five gallons per ton of fresh ensilage material. Tap water was added to the control silage in the same volume as the urea solution was to the experimental silage.

The urea corn silage was of fair quality, possessing a slightly caramelized odor and a brownish color. There was no free ammonia, as detected by the sense of smell, except in the spoiled surface layer at the top of the silo.

Temperatures at the center of the silos, from 24 to 28 inches below the surface of the silages, were the same for the treated and the untreated. The sub-surface temperatures were different but variable and inconsistent. The significance of these variations has not been established.

As indicated by conventional feed analysis the only notable difference in the dry matter content of the fermented silages was in the "crude protein," which was 10.79 per cent for the treated and 7.48 per cent for the untreated. Average carotene values per gram of dry matter were 11.50 micrograms and 36.58 micrograms for the treated and untreated, respectively. The corresponding pH values were 4.3 and 3.6.

The palatability of the two silages and their comparative effects on carotene values of the milk, on milk production and on the weights of the cows were investigated by means of a double reversal feeding trial, which was conducted over three 42-day periods using two groups of 11 cows each. The respective silages, which were the only roughages in the rations, were fed slightly in excess of the amounts the individual cows consumed. The concentrates, fed at the same level in both groups, were adjusted according to the milk production of all the cows in the test.

The palatability of the treated silage was somewhat low; the average daily consumption per cow was 52.5 pounds (15.5 pounds of dry matter); whereas that for the untreated was 60.0 pounds (16.9 pounds of dry matter).

The effects of differences in the carotene values of the silages were reflected in the carotene concentrations of the milk; the average per liter of four per cent fat-standardized milk was 153.5 micrograms for the treated and 258.6 micrograms for the untreated.

The productive values of the two silages were similar. The average daily production of four per cent fat-corrected milk per cow was 24.7 pounds for the treated and 24.5 for the untreated. The average total gains in weight per cow for the trial were 43 pounds for the treated and 56 pounds for the untreated.

These data indicate that treating corn silage with urea does not improve the over-all feeding value of the resulting silage.

### **P33. The Minimum Protein Requirements of Young Holstein Calves.**

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Calves were reared to eight weeks of age according to the whole milk-dry

starter system used by Savage and Associates. From the eighth to the sixteenth week they were fed diets containing 8.3, 11.7, 15.2, and 18.8 per cent crude protein (dry basis). Nitrogen balances were run on the males at approximately the tenth and fifteenth week. The diets included only plant proteins and were balanced in energy, fat, calcium and phosphorus content.

Heifers fed the 18.8 per cent protein diet ad libitum were slightly larger than normal (Ragsdale). Diets containing 15.2 and 11.7 per cent of protein permitted a growth rate slightly below normal. Bull calves on a somewhat limited intake of food were all below normal in rate of gain. There was no apparent difference in the gains of the males receiving diets containing 18.8 and 15.2 per cent protein. The diet with only 8.3 per cent protein gave very poor growth.

About the same amounts of nitrogen were stored by calves fed the diets containing 15.2 and 18.8 per cent protein but the other two diets gave appreciably less storage of nitrogen. Endogenous and metabolic nitrogen determined on some of the calves when they averaged 97.2 kgm. in weight and 137 days of age, were 0.041 gm. per kgm. (1.999 gm. per  $M^2$ ) and 0.372 gm. per 100 gm. dry matter consumed, respectively.

**P34. Studies on Carotene Conversion in the Young Calf.** NORMAN S. LUNDQUIST AND PAUL H. PHILLIPS, *University of Wisconsin, Madison, Wis.*

Calves fed beta carotene as the only source of vitamin A from birth failed to survive in 75 per cent of the cases, whereas those fed vitamin A failed to survive in only 40 per cent of the cases. When vitamin A *per se* was fed, blood plasma levels of 9 to 11 micrograms per 100 ml. were reached in 8 to 10 days, whereas with carotene these levels were not reached until the 20th day. Blood plasma levels were maintained in the calves receiving either 18 or 90 micrograms per kg. body weight of vitamin A. Calves getting vitamin A *per se* did not reach plasma carotene levels above 4 micrograms per 100 ml. during the 4 weeks of the experiment. Calves fed the carotene showed a concentration of 11 micrograms per 100 ml. during the first week, after which a rather wide variation occurred with a maximum of 17 micrograms per 100 ml. blood plasma.

There was no observable difference in blood-plasma ascorbic acid between the groups. The blood-plasma vitamin-C curve was nearly identical to that reported earlier for calves on a skimmilk diet and getting vitamin C *per os*. Administration of 2 grams daily of succinyl sulfathiazole had a slight effect in maintaining higher blood-plasma ascorbic acid during the first week. Blood-plasma ascorbic acid levels of calves at death was 0.49 mg. per cent, indicating death was not due to an active infection. However, all calves which died had 5 micrograms or less of vitamin A per 100 ml. blood plasma at death.

**P36. The Effect of Sulfa Drugs and Immunity Development Upon Blood-Plasma Vitamins A and C in the Young Bovine.** NORMAN S. LUNDQUIST AND PAUL H. PHILLIPS, *University of Wisconsin, Madison, Wis.*

During the first two weeks of the life of the newborn calf, succinyl sulfathiazole, when fed at the low level of 2 grams per day, resulted in higher blood-plasma ascorbic acid levels than in untreated calves. This drug did not appear to have any effect on blood-plasma vitamin-A or carotene levels. Sulfapyridine or succinyl sulfathiazole had no effect in increasing blood-plasma ascorbic acid above normal in young Holstein heifers. Sulfathiazole, however, caused a very definite increase. This effect was somewhat similar to that caused by the feeding of chlorobutanol. When the latter two drugs were given consecutively, a marked reduction in blood-plasma ascorbic acid occurred. The addition of sulfathiazole or succinyl sulfathiazole to the diet did not interfere with the destruction of vitamin C in the rumen, since no increased effect was noted in blood plasma levels, even when this vitamin was also fed.

Blood-plasma ascorbic acid and vitamin-A levels were temporarily reduced by calfhooed vaccination, irrespective of additions of these vitamins to the normal diet. A rise in rectal temperature followed closely the drop in blood-plasma ascorbic acid. The leucocyte count which increased on the day following inoculation returned to normal in five days. Differential count showed that the neutrophils were increased, the lymphocytes decreased, and monocytes and eosinophiles not materially affected. The calves when vaccinated were normal with respect to vitamin nutrition and did not develop deficiency symptoms.

**P37. The Use of Stored Colostrum to Replace Marketable Milk for Calf Feeding.** N. N. ALLEN, *Vermont Agricultural Experiment Station, Burlington, Vt.*

Milk is one of the most expensive items in the feed cost of raising calves, even if the amount fed is kept at a minimum, and in aggregate this accounts for a large volume of milk. With present high prices and wartime demands, this takes on additional aspects of importance.

Colostrum is unmarketable and is usually produced in excess of the immediate needs of the calf. This surplus is often wasted or used to poor advantage. If it could be held until needed, it has possibilities in replacing salable milk for feeding calves beyond the age of normal colostrum feeding. It has repeatedly been shown that colostrum may be stored in frozen condition without seriously affecting its nutritional properties. Because of its bulk, the cost is relatively high if artificial refrigeration is used. In the northern parts of the country natural refrigeration may be used during the winter months. In the New England area a large portion of the cows are



bred to freshen during the months when winter temperatures permit freezing and frozen storage without mechanical refrigeration. On most farms in this area less than a third of the calves are raised and if the total colostrum production were utilized, it might replace most of the milk now used for calf feeding.

At the Vermont Experiment Station, some of the practical possibilities of using colostrum for this purpose are being studied.

Three plans have been followed and compared. Under one plan, the calves serving as a control group received the fresh colostrum or milk from their dams as produced during the first ten days. Under the second plan, the calves received colostrum stored in frozen condition, during the first ten days. After ten days, the calves of both groups received fresh mixed milk from the herd until 24 days of age when they were changed (with a three-day transition period) to skimmilk which was fed until they reached 63 days of age.

Under the third plan, the calves received stored colostrum, with no other milk until 63 days of age.

No serious digestive disturbances occurred in any of the calves. The calves of all three groups made very satisfactory gains. The control group lost weight between the second and fourth days, probably due to the change in composition of the mothers' milk at that time, while the colostrum-fed groups did not. As a result, the growth curve of the control group was slightly below that of the colostrum-fed groups after the second day, but the rate of gain was essentially the same. The calves receiving colostrum throughout the milk-feeding period were fatter and sleeker of coat, showing the characteristics of whole-milk-fed calves. Two slaughtered at 9 weeks of age showed good finish, and made veal of very good quality.

Some of the colostrum fed had been in storage for over a year. During the winter months colostrum was frozen and stored for periods of more than two months without artificial refrigeration. It was shown clearly that colostrum stored in frozen condition is entirely satisfactory as a substitute for marketable milk for feeding calves during any part of the milk-feeding period.

#### **P38. Experiences with the Forage Harvester in Making Grass Silage.**

C. F. MONROE, *Department of Dairy Industry, Ohio Agricultural Experiment Station, Wooster, Ohio.*

For the past two seasons a forage harvester\* has been used to cut and chop green material in the field for grass silage. This machine can chop the material as it stands in the field or after it has been allowed to partially dry in the windrow or swath. The experimental work has been concerned chiefly with ensiling the crops without wilting. Since such material may

\* In cooperation with the Allis-Chalmers Co., Milwaukee, Wis.

contain an excess of moisture, emphasis has been placed upon the use of dry matter increments, such as ground shelled corn, ground ear corn, and beet pulp. With these treatments acceptable grass silage has been made using the forage harvester. For measuring the quality of the silage, palatability trials have been conducted with dairy cows and heifers, beef cows, and also sheep. The forage harvester offers a means of reducing the labor and irksomeness of making grass silage.

**P39. Development and Use of the Barn Haydrier.** C. E. WYLIE, *Tennessee Agricultural Experiment Station, Knoxville, Tenn.*, AND JOHN A. SCHALLER, *Agricultural Engineer, Tennessee Valley Authority.*

For several years agricultural workers have realized that a limiting factor in the production of high quality hay in the southeast is the difficulty of curing it. With about 50 inches of rainfall a year, the southern states have some of the nation's best hay GROWING weather and some of the worst hay CURING weather.

Through several years of research, beginning in 1935, a method of barn curing of hay was developed jointly by the Tennessee Valley Authority and the Tennessee Agricultural Experiment Station, involving low first cost and low operating cost to complete the curing of hay in the barn after taking advantage of several hours of field curing. The University of Georgia conducted studies from 1937 to 1940 on the adaptation of this method to the climatic and operating conditions in Georgia. Virginia Polytechnic Institute began similar studies in 1939, which also covered the use of artificial heat.

Briefly, the hay drier consists of specially constructed air ducts on the haymow floor. Partially dried hay (40 to 50 per cent moisture content) from the field is stored as usual over the air ducts. A blower or fan, either the centrifugal or propeller type driven by an electric motor or gasoline engine, connected to the air ducts underlying the hay, forces air up through the hay and removes the moisture.

Past experience has definitely shown that with a barn haydrier a farmer can do two things: (1) eliminate some of the weather risk in haymaking and (2) make a superior quality of hay with a total cost of \$1.40 to \$1.65 per ton.

Quality studies show barn-dried hay to be consistently better by at least one grade than similar field-dried hay. The price differential on the southern markets between U. S. No. 1 and U. S. No. 2 range from \$2.40 to \$3.00 in peacetime and is even greater during wartime. The gain then to the farmer would be \$1.00 to \$1.50 per ton of hay plus the gain in weight of hay.

Feeding trials with barn-dried hay have been conducted at the Tennessee Experiment Station with dairy cattle and other livestock. In general, more attention has been given to developing and improving the method of drying

the hay than to checking the feeding value of the hay. The improvements in the method of drying have been rapid from year to year and still are subject to further improvement.

During wartime when protein feeds are scarce and every ounce of feeding value in the hay should be saved, the importance of the barn haydrier is even greater. The War Production Board has allocated materials for 300 installations this season in the Southeastern states in addition to materials for other states.

**P40. Congenital Muscular Contracture and Ankylosis in Jersey Cattle.**

A. A. SPIELMAN, O. J. HILL, AND E. C. McCULLOCH, *State College of Washington, Pullman, Wash.*

The occurrence of a large number of calves with congenital deformities in several registered Jersey herds of similar breeding is being investigated. To date, records of 69 cases have been accumulated.

Although considerable variation occurs in the appearance of these anomalies, both lethal and sublethal, the gross manifestations appear to be varying degrees of muscular contracture and ankylosis. Deformities of the extremities, mainly the forelegs, are characteristic of all the cases. In addition, some of the abnormal calves have a wry neck, flexure of the spinal column and incompletely developed palate. The extent of these deformities in many cases caused severe dystocia, necessitating either dismemberment or Caesarian delivery.

A fairly close degree of inbreeding of identical bloodlines has been practiced in all the herds where these abnormalities have been observed. Although the malformations observed in these cases are similar to the muscular contracture condition described by Löje, Williams and Hutt as being hereditary, final etiological judgment is withheld until further investigation because unfavorable prenatal nutrition and environment may also cause congenital deformities.

**P41. The Optimum Emphasis on Dams' Records when Proving Dairy Sires.** JAY L. LUSH, *Iowa State College, Ames, Ia.*

Nearly all proposals for evaluating the proof of dairy sires are special forms of the general equation  $I = a + c(X - bY)$  in which  $I$  is the index or other measure of the bull's breeding value,  $X$  is the average production of his daughters,  $Y$  is the average production of the dams' of those daughters and  $a$ ,  $b$  and  $c$  are constants which define the kind of index being used. This paper shows what value of  $b$  will make the correlation between  $I$  and the real breeding value of the sire as high as it can possibly be for any index of the general type described by the above equation. If the sire's breeding value is not correlated with  $Y$ , the optimum value of  $b$  is the regression of

X on Y which is between 0.5 and 0.6 in most dairy data as actually used for proving sires. If the sire's breeding value is slightly correlated with Y, as might result from assortive mating, the optimum value of  $b$  is a little smaller than this regression of Y on X. The widely used "intermediate" or "equal-parent" or "modified Mount Hope" index uses 0.5 for  $b$ .

The values of  $a$  and  $c$  do not affect the *accuracy* of the index but may make it easier or harder to understand, more susceptible or less susceptible to misinterpretation, etc. Changing  $c$  will expand or contract the variability of the index. Changing  $a$  will affect only the general level or average of the indexes. The most satisfactory value for  $c$  is that which will make the indexes of sires about as variable as either (1) the records of cows or (2) the most probable breeding values of the sires. There are some arguments in favor of each of these alternatives.

**P42. Comparison of the Incidence of Udder Infection in Two Cow Families.** JAMES M. MURPHY, K. O. PFAU, O. L. LEPARD, AND J. W. BARTLETT, *New Jersey Agricultural Experiment Station, New Brunswick, N. J.*

The occurrence of udder infection was studied for a period of six years in two cow families living under the same conditions in a herd of 60 milking cows. The one family was composed of the dam (283), three daughters, and two granddaughters. The other family was composed of the dam (247), four daughters, and two granddaughters. Of the former family, seventeen lactation periods were studied: four *first*, four *second*, three *third*, three *fourth*, and three *fifth*. Of the latter family, eighteen lactation periods were studied: five *first*, four *second*, three *third*, three *fourth*, one *fifth*, one *sixth*, and one *seventh*.

In evaluating the incidence of infection, a system was devised whereby the infection present in each quarter could be stated in terms of the percentage of the lactation period during which it was present. By this method it was found that family 283 was infected with streptococci during 23.9 per cent of its milking time and by staphylococci during 39.3 per cent of its milking time. The total incidence of infection was 63.3 per cent. Family 247, on the other hand, was found to be infected with streptococci during 0.9 per cent, and with staphylococci during 10.9 per cent, of its milking time. The total incidence of infection was 11.8 per cent.

The mastitis history (considered entirely separately from infection which was determined bacteriologically) of the two families was judged by periodic leucocyte counts of strict foremilk, daily strip-cup examination of foremilk, and periodic physical examination of the udders. Family 283 showed a leucocyte content of 500,000 or more per cc. in 179 (42.1 per cent) of 425 samples of strict foremilk, showed the presence of flakes or more marked alteration of the secretion at 454 daily strip-cup examinations, and averaged

No. 3 by the Udall system of udder physical examination. Family 247 showed a leucocyte content of 500,000 or more per cc. in 55 (10.3 per cent) of 536 samples of strict foremilk, showed the presence of flakes or more marked alteration of the secretion at 192 daily strip-cup examinations, and averaged No. 2 by the Udall system of udder physical examination.

**P43. The Effect of the Oral Administration of Chloreitone to Bulls on the Fertility of the Semen Produced.** IRVINE ELLIOTT, *Cornell University, Ithaca, N. Y.*

Six mature bulls, 3 Holstein and 3 Guernsey, all in active service through artificial insemination, were fed 5 g. daily doses of "Chloreitone." The bulls were not "slow breeders" but had been producing semen low in spermatozoa concentration, percentage progressive motility, and fertility.

The "Chloreitone" was fed for 8 days, discontinued for one week, and again fed for 21 days. The level of ascorbic acid was increased as much as 100 per cent in the blood plasma of the bulls following treatment. The ascorbic acid content of the semen was also increased, but no other effect on semen quality was noted.

**P44. Storage of Bovine Spermatozoa in Diluents Containing Certain Amino Acids (Progress Report).** C. E. KNOOP AND W. E. KRAUSS, *Ohio Agricultural Experiment Station, Wooster, Ohio.*

The accumulation of laboratory and breeding data has shown that the egg-yolk-gelatin buffer diluent for bovine spermatozoa is reliable for maintaining fecundity of average to excellent quality spermatozoa after 6 to 14 days in storage. Sperm cells stored in this diluent maintained an average of 20 per cent more living cells after 8 to 10 days than when no gelatin was used. An attempt is now in progress to explain this difference by first removing the gelatin and adding some of the major amino acids found in gelatin. Criteria used to date have been hydrogen ion content, motility rating, and breeding record.

The first two amino acids studied have been glycine and proline plus hydroxyproline because they represent 25.5 and 23.6 per cent, respectively, of gelatin protein. The hydrogen ion content of the buffered portion of the amino acid diluents was approximately 7.20 pH and of the completed diluent approximately 6.30 pH.

Results show that a high percentage of living sperm cells was maintained in storage for 2 weeks in a buffered portion of diluent containing 1.09 per cent of glycine or "1"-proline, 0.2 per cent sodium phosphate, and 0.08 per cent potassium phosphate mixed with an equal volume of fresh egg yolk. On the average, the amino acid diluents maintained 36 and 49 per cent more living cells after 12 days in storage than did the egg-yolk-gelatin and egg-

yolk diluents, respectively. The rate of motility also remained good in two-thirds of the diluted samples containing glycine or "1"-proline.

**P45. Recent Observations on the Preservation of Bull Semen.** HENRY A. LARDY AND PAUL H. PHILLIPS, *University of Wisconsin, Madison, Wis.*

Chemical studies on the phosphorus partition of bull spermatozoa indicates that adenosine triphosphate (or a chemically similar compound) is present in bull spermatozoa in appreciable quantities. The enzyme adenosine triphosphatase which breaks down adenosine triphosphate to adenylic acid and inorganic phosphorus is likewise present. A second enzyme has been separated from the adenosine triphosphatase by acetone treatment and has been shown to be specific for the hydrolysis of adenosine-5-phosphate yielding inorganic phosphorus. These results indicate a great similarity in the energy metabolism of striated muscle and spermatozoa, and attempts have been made to apply this knowledge to the storage of semen for artificial insemination.

When methods of arresting muscle contraction were applied to spermatozoa the results were variable. Attempts at ion displacement of the activators of adenosine triphosphate did not result in inactivation of the spermatozoa. The only procedure which was found to allow reversible inactivation of the spermatozoa was treatment with fluoride. Some specimens could be inactivated for periods up to 7 days and following centrifuging and dilution of the spermatozoa with fresh yolk-buffer vigorous motility was resumed.

The relation of the enzyme hyaluronidase to fertility of bull semen has also been investigated and will be discussed.

**P46. Further Studies of the Effect of Dilution Rate on the Fertility of Bull Semen Used for Artificial Insemination.** G. W. SALISBURY, IRVINE ELLIOTT, AND N. L. VANDEMARK, *Cornell University, Ithaca, N. Y.*

In a continuation of studies previously reported (JOUR. DAIRY SCI., 26: 1057-1069, 1943) an investigation has been made into the effects of increased dilution of bull semen upon its fertility when used for routine artificial insemination. When insemination was made deep into the uterus with 1.0 cc. or less of diluted semen no difference in fertility was found between dilution rates of 1 part of semen to 8, to 12, to 16, to 24, or to 50 parts of the yolk-citrate diluter. No difference in quality was found for the semen used at the several dilution rates as indicated by spermatozoa count, initial motility, and methylene blue reduction time. No semen was used which contained less than 1,000,000 spermatozoa per mm.<sup>3</sup> or which exhibited less than 50 per cent

of actively motile spermatozoa. With the highest rate of dilution, 1:50, about 26 million spermatozoa were used per insemination. 3,296 inseminations were made during the investigation.

**P47. Conception Rate in Dairy Cattle by Artificial Insemination at Various Intervals Before and After Ovulation.** G. W. TRIMBERGER, *University of Nebraska, Lincoln, Nebr.*

A total of 132 females of the Ayrshire, Jersey, Guernsey, and Holstein breeds in the University of Nebraska dairy herd were checked at two-hour intervals during and following the heat period to determine the end of estrus and time of ovulation on the average within a time interval of one hour or less. Females were considered in heat if complacent when mounted and the time of ovulation was determined by palpation of the ovaries per rectum through the intestinal wall. The rupture of the follicle on the ovary indicated ovulation.

The females were bred at various time intervals before and after ovulation to determine the effect of this factor on breeding efficiency. Services before ovulation were grouped as follows: More than 24 hours before rupture of the follicle, from 19 to 24, 13 to 18, 7 to 12, and 6 hours or less before ovulation. Three groups of females were bred after ovulation with 20 in each one of the following groups: 2 hours or less after ovulation, 6 hours, and 12 hours after rupture of the follicle. All services were by artificial insemination with semen of high quality from bulls with a good breeding record. Semen samples used were stored from one to three days or more as is a common practice in cooperative artificial breeding associations. For these inseminations 0.25 cc. of semen diluted 1:3 with egg-phosphate buffer or a total of 1 cc. diluted semen was used and the semen was deposited in the uterine horn on the side where the ovary had developed the follicle.

Under practical conditions when the females were observed three times daily the duration of estrus had a range from 2½ to 28 hours and averaged 16.92 hours (86 cows averaged 17.77 hours and 46 heifers averaged 15.33 hours). Ovulation time after end of estrus had a range from 3 to 18 hours with an average of 10.49 hours (86 cows averaged 10.66 hours and 46 heifers averaged 10.16 hours).

Breeding efficiency, from one insemination, of the females in the various groups is tabulated below:

<i>Time of breeding</i>	<i>No. females</i>	<i>Conceptions</i>	
		<i>No.</i>	<i>%</i>
More than 24 hours before ovulation .....	15	8	53.33
From 19 to 24 hours before ovulation .....	15	11	73.33
From 13 to 18 hours before ovulation .....	14	12	85.71
From 7 to 12 hours before ovulation .....	14	11	78.57
Six hours or less before ovulation .....	14	8	57.14
Two hours or less after ovulation .....	20	6	30.00
Six hours after ovulation .....	20	8	40.00
Twelve hours after ovulation .....	20	6	30.00

Although there are no significant statistical differences among the groups bred before ovulation, there is a trend toward a higher rate of conception in females bred from 6 to 24 hours before ovulation, which coincides with a previous study in which the highest rate of conception was obtained in the middle and toward the end of the heat period. A total of 72 females were bred in the various groups before ovulation and 50, or 69.44 per cent, conceived. Only 20, or 33.33 per cent, of the 60 females bred 12 hours or less after ovulation conceived, and this indicates that the bovine ovum in many cases remains fertile only a relatively short time after ovulation.

To provide information useful for breeding cows in cooperative artificial breeding associations, the females were tabulated as to the time estrus was first observed. Eighty-one, or 61.36 per cent, of the females were in heat in the forenoon and 51, or 38.64 per cent, were first observed in heat in the afternoon. Females observed in heat in the forenoon averaged 15.57 hours for duration of estrus as compared to 19.07 hours for females first observed in heat during the afternoons. The latter represents about the normal time for the heat period, for the females in this group were detected soon after the start of estrus. The time heat was first observed was then compared with the time of ovulation on the following day. Breeding results can be predicted based on the assumption that females bred before ovulation have a good chance for conception and those bred after ovulation will have a poor conception rate. The practical question involved is what conception rate can be obtained in cows bred the day following estrus if these inseminations are either in the forenoon or in the afternoon. Results from 81 cows in heat in the forenoon indicated that 31, or 38.27 per cent, had ovulated before 8:00 A.M. the next day. Another 16, or 19.75 per cent, ovulated during the forenoon and 30, or 37.04 per cent, ovulated during the afternoon. Only 4, or 4.94 per cent, ovulated after 6:00 P.M. of the day following estrus.

Among the 51 females which were first observed in heat in the afternoon, none had ovulated before 8:00 A.M. the next morning and only 2, or 3.92 per cent, ovulated during the forenoon. Seventeen, or 33.34 per cent, ovulated in the afternoon before 6:00 P.M. and 32, or 62.74 per cent, ovulated after 6:00 P.M.

Since breeding efficiency is high for females inseminated before the follicle is ruptured and low for those bred after rupture of the follicle, the females in heat during the early forenoon should be bred on the same day. Females coming into estrus in the afternoon can be bred any time during the following day with good results, but there is some advantage to breeding such cows in the forenoon or early in the afternoon. If a farmer in a cooperative breeding association carefully observes his cows, he can report females in heat in the forenoon for service on the same day, and those which come in heat in the afternoon can be reported the next morning and bred during the forenoon or early afternoon with good results.



**P48. The Methylene Blue Reduction Test and Its Relation to Other Measures of Quality in Bull Semen.** G. W. SALISBURY, ERNEST MERCIER, AND N. L. VANDEMARK, *Cornell University, Ithaca, N. Y.*

In an experiment involving 116 separate ejaculates of bull semen the correlation between the methylene blue reduction test (*JOUR. DAIRY SCI.*, **26**: 483-494. 1943) and several other characteristics concerned in measuring semen quality was determined. Correlations were calculated using individual ejaculates so that the results would be applicable for prediction with respect to any single semen sample. The semen ejaculates in the study represent collections from 39 individual bulls during 6 different months throughout the year. Marked differences were found in the degree of association between certain characteristics at the various seasons represented.

The measured characteristics which were not significantly correlated with the methylene blue test included the proportion of abnormal spermatozoa, the ascorbic acid, glucose, and lactic acid levels of fresh semen, and the glucose loss in diluted semen stored 10 days at 5° C. The test was significantly correlated with pH in a positive direction and with volume in a negative direction.

However, the methylene blue test held a highly significant negative correlation with livability, glucose loss, and lactic acid gain in diluted semen after an hour's incubation at 46.5° C. Similar highly significant negative correlations also were found with livability and lactic acid gain in diluted semen stored for 10 days at 5° C. The high degree of association between the methylene blue reduction time and the products involved in glycolysis, the original count and motility, as well as its close relationship with livability indicates that the test is actually a rapid method of measuring the potential ability of spermatozoa to continue glycolysis and live.

**P49. The Effect of Pregnancy on the Body Weight of Dairy Cows.** D. N. PUTNAM AND H. O. HENDERSON, *University of West Virginia, Morgantown, W. Va.*

Normal growth curves have been developed for all the breeds of dairy cattle. These are usually measured by the weight of the animals and by the height at withers. While pregnancy does not greatly affect the height of withers it does affect the weight of the animals. After the animal is bred the weight curve takes an upward turn, and this curve is no longer a measure of the growth, but includes also the weight of the fetus and accompanying material, including the fetal membrane and amniotic fluid.

To determine how much this curve was affected by pregnancy a study was made at the West Virginia Agricultural Experiment Station with 56 normal Ayrshire females that had calved at least three times. The same animals were used throughout the study. The average age for first calving

was 32 months; for second calving, 46 months; and for the third calving, 59 months.

The following results were observed: (1) The body weight shows a definite pattern for increase as the stage of pregnancy advanced. (2) The gain in weight over and above normal weights was not great before the fifth month of pregnancy. From 75 to 85 per cent of the gain came in the last four months of pregnancy. (3) The gain in weight from month to month was much more uniform with first calf heifers than with the older animals. This was probably because the heifers were still growing and not producing milk. (4) In every case, the animals gained less on the average during the eighth month of pregnancy than they did in the preceding and following months. (5) The animals in the last month of pregnancy were on the average 139 pounds heavier than open heifers of the same age. During the first pregnancy they were 115 pounds heavier; the second pregnancy, 147 pounds heavier; and the third pregnancy, 155 pounds heavier.

**P50. Factors to Consider in Long-Distance Shipping of Semen.\*** H. A. HERMAN AND ERIC W. SWANSON, *University of Missouri, Columbia, Mo.*

The shipping of dairy bull semen to distant points occupies an important place in fully utilizing sires of superior breeding for artificial insemination purposes. There are three very important factors affecting the success of long-distance semen shipping: (1) A sire capable of producing spermatozoa with an inherent capacity for long-time survival under storage conditions; (2) proper handling and packaging of the semen in a container which will maintain temperatures of 35 to 50° F. until delivery; and (3) proper evaluation and use of the semen upon arrival at its destination.

The capacity of semen to withstand storage is usually one of the limiting factors affecting the success of this program. At least 40 per cent of the sires studied at the Missouri Station have yielded semen unsatisfactory for shipment to distant points, though many such bulls would be satisfactory if the semen was utilized within 6 to 20 hours. In a study of 475 inseminations using stored semen it was found that as viability of the sperm increased the conception rate increased until 68 per cent of the inseminations with the most viable semen resulted in conception. A highly significant linear correlation (0.84) was found between viability of sperm in storage and their ability to produce fertilization. The motility rating of semen may give a good insight into its capacity to produce conception, and in 565 conceptions an index of correlation of 0.97 was obtained. The difference in conception rate between semen rated 3 motility (45 per cent or more progressively motile sperm) and higher grades of motility was slight. Semen

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containing less than 20 to 45 per cent progressively motile sperm was not very satisfactory for storage and shipping as only 43 per cent conceptions resulted from its use. The correlation between pH, abnormal sperm, and concentration of sperm is generally of low significance as compared to the importance of (1) ability to withstand storage and (2) the motility rating.

At the Missouri Station a small insulated ice cream shipper fitted with a metal can to contain crushed ice and a vacuum bottle has been found to be very satisfactory for long-distance shipping. Semen temperatures of 35 to 50° F. have been maintained in this container for 84 hours at atmospheric temperatures of 80° F. Less complex shipping containers are satisfactory for intra-state shipments.

Using the above technique, semen shipped from Missouri to New Jersey has resulted in 20 pregnancies from 42 inseminations (semen 60 to 70 hours old when used). Additional shipments from Missouri to Indiana, and from Oklahoma to Missouri have resulted in 25 pregnancies from 47 inseminations, giving an average of 1.98 inseminations per conception for all shipments.

## MANUFACTURING SECTION

### M1. Factors Which Influence the Apparent Survival of Heat-Treated Bacteria. F. E. NELSON, *Iowa State College, Ames, Ia.*

The quantitative effects of variations in medium and temperature of incubation employed in making plate counts on dairy products commonly are attributed to the establishment of conditions such that the numbers of species or strains of bacteria which are able to produce countable colonies is changed. While this explanation may be adequate under some circumstances, results previously reported, using pure cultures of heat-treated bacteria, have shown that numbers of colonies can be influenced markedly by the plating medium employed. The results of certain studies of quantitative and qualitative changes in composition of the plating medium, of certain methods of combining the ingredients, of pH of the medium and of time and temperature of incubation upon plate counts of heat-treated bacteria are reported in this paper. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus durans*, *Streptococcus liquefaciens* and *Streptococcus zymogenes* were used as test organisms. Milk or broth cultures of these organisms were diluted with sterile skim milk and heated to such temperatures for such periods of time as would prevent proliferation of the majority of the organisms under usual cultural conditions.

Most of the factors studied were without effect in the ranges employed upon the development to countable colonies of the unheated control bacteria; counts of heat-treated bacteria were affected, sometimes quite markedly, by modifications in the cultural conditions. Larger quantities of pep-

tone were more effective than small quantities in inducing development of maximum numbers of colonies. Addition of peptone to the basal medium before sterilization resulted in higher counts than did addition of a sterile peptone solution in equivalent quantity at the time the plates were poured, except when very small quantities were employed. Addition of thioglycollic acid to various media commonly resulted in rather large increases in count, a fairly definite optimum concentration of this substance being observed for each medium in the cases of the majority of the test organisms. A comparatively narrow pH range of the counting medium proved optimum for development of maximum numbers of colonies of heat-treated bacteria. This optimum was particularly pronounced with *E. coli* and *Ps. aeruginosa*, where it occurred at about pH 5.7. An incubation temperature of 32° C. proved optimum or nearly optimum for maximum count on most suspensions of heat-treated bacteria, with 28° and 37° C. being slightly less satisfactory in most instances and 42° C. being particularly unsatisfactory. Incubation at 21° C. commonly was very unsatisfactory when only 2 days were allowed for incubation, but incubation for 4 days greatly improved the results at this temperature, as well as improving the count level appreciably at 28° C. and to a smaller extent at 32° C. At 37° and 42° C. the longer incubation period had essentially no effect upon the count.

The data indicate all of the factors studied have an appreciable effect upon the apparent survival of bacteria subjected to sublethal heat, while being essentially without effect upon colony development of unheated control organisms from the same culture. Undoubtedly some of the observed differences in counts on pasteurized dairy products resulting from modifications of culture media and incubation conditions are due to the effect of these factors upon the ability of heat-treated bacteria to produce countable colonies, rather than because of growth of new strains or species of bacteria.

**M2. A Correlation of the Resazurin Test Read with an All-Purpose Lovibond Comparator at 10, 30, and 60 Minutes with the Standard Plate Count of Milk.** S. IRENE JORGENSEN AND N. S. GOLDING, *Washington Agricultural Experiment Station, Pullman, Wash.*

Extensive work has been done by the dairy research stations in Great Britain on the resazurin test for estimating the quality of farmer's milk. The color changes in this test have been standardized by a special Lovibond Comparator giving discs from blue, lilac, mauve, pink-purple, purple-pink, pink and white which are numbered 6, 5, 4, 3, 2, 1 and 0, respectively, and graded down from good to poor. The Ministry of Agriculture and Fisheries of Great Britain are recommending the resazurin test as a standard routine test and have issued a provisional technique for operating the test.

In our work to correlate the resazurin test with the Standard Plate Count (Standard Methods for the Examination of Dairy Products, American

Public Health Association, 8th Edition, 1941) we have used some 1360 samples of farmer's milk taken on the Portland, Oregon milk-shed in April and May, 1944. Standard plate counts were made by the Interstate Dairies in their laboratory and with their kind cooperation we obtained milk from each of these samples and made 30- and 60-minute resazurin readings on the comparator concurrently with their plate count.

Frequency curves for each resazurin disc number are plotted against the log of the standard plate count for thirty and for sixty minutes.

Previous work done during last winter in our laboratory including a smaller number of samples is expressed in a similar way for resazurin readings at 10, 30 and 60 minutes.

**M3. Mastitis and the Plate Count.** E. O. ANDERSON, W. N. PLASTRIDGE, AND H. W. SEELEY, JR., *Storrs Agricultural Experiment Station, Storrs, Conn.*

Our results seem to indicate that in general high bacterial counts in milk are due to causes other than the chronically infected cow's udder. A previous report showed that *Streptococcus agalactiae* counts in 33 samples of mixed herd milk averaged 1,100 bacterial colonies per ml. Recent work shows that the *Streptococcus uberis* population of 23 samples studied ranged from 30 to 40,000 bacterial colonies with an average of 8,200 per ml. Samples of milk drawn from 34 staphylococcus-infected quarters gave plate counts ranging from 200 to 42,000 staphylococcal colonies per ml. with an average of 6,800. Representative portions of the complete milking from seven chronically coli-infected quarters were plated on violet red bile agar and the plate counts were found to be less than 100 colonies per ml.

The region of 50° F. is critical for the growth of *Str. uberis* in milk. A statistical analysis disclosed that at this temperature 10 of 15 cultures showed a slight measurable increase in numbers during a 60-hour incubation period. Four of five cultures of *Str. agalactiae* did not grow at 50° F. under the same conditions.

**M4. Behavior of Micro-Organisms in Fluid Dairy Products When Held in Atmosphere of Different Gases under Pressures.** M. J. PRUCHA, *University of Illinois, Urbana, Ill.*

In this study milk and cream, both raw and pasteurized, were subjected to carbon dioxide, nitrous oxide and freon at different pressures and temperatures. Plate counts were made to determine the rate of multiplication of the bacteria present.

It was found that such treatment greatly reduced the rate of multiplication of bacteria. For example, in one test milk having a plate count of 10,000 and held at 50° F. had a count after four days of 31,000,000 while

the same milk subjected to 55 pounds pressure of nitrous oxide had a count of only 61,000.

In another test milk was inoculated with heat-resisting *E. coli* and was pasteurized at 130° F. for thirty minutes. The initial count was 7,000,000. The lot subjected to 100 pounds pressure of nitrous oxide was free from any *E. coli* while the lot not put under the pressure of the gas had a count of 44,000.

The effect of freon was about the same as that of nitrous oxide. The effect of carbon dioxide was more pronounced. When milk was subjected to air pressure and was then pasteurized, the pressure of 100 pounds also tended to cause a greater reduction of the number of bacteria by pasteurization.

A tentative conclusion of this study may be drawn to the effect that a combined effect of two or more harmful factors on microorganisms applied at the same time causes greater destruction of the microorganisms than the factors applied singly. This principle may become important in food preservation.

**M6. Farm Sources of *Oospora lactis*.\*** E. R. GARRISON, *University of Missouri, Columbia, Mo.*

Qualitative determinations were made to detect the presence of *Oospora lactis* in dairy farm milk utensils and in various miscellaneous materials collected on farms. Quantitative determinations of this organism were usually not attempted because of the abundance of other molds in most materials examined, which overgrew and obscured the *Oospora lactis* colonies on the agar plates.

A portion of the inner surfaces of the milk utensils on 60 cream-producing farms were wiped, first with a moist, then with a dry cotton swab and the swabs and water (20 ml.) used were plated on acidified potato-dextrose agar and after incubation at room temperature the plates were examined for *Oospora lactis* colonies. Of the 60 milk pails, 23 milk strainers and 36 cream separators examined, 76.7, 65.2 and 80.6 per cent, respectively, contained *Oospora lactis*. Other molds were present, usually in relatively large numbers, on all equipment studied. The rubber parts of 20 milking machines were flushed with 500 ml. of sterile milk and the milk collected in a liter flask; after inoculating with butter culture, the flasks were held at a favorable temperature and observed for the growth of *Oospora lactis* in the cream on the surface of the milk; the rubber parts of only one machine yielded this organism.

Eighty-one samples of cistern water used for washing the milk utensils on dairy farms were analyzed for *Oospora lactis* by plating 1- and 5-ml.

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quantities in acidified potato-dextrose agar; this mold was found in 27 or 33.3 per cent of the water samples investigated.

The presence of *Oospora lactis* in the barn air was studied by exposing two sterile Petri dishes for 15 minutes at various places in 30 dairy barns under both clean and dusty conditions. One plate was later poured with acidified potato-dextrose agar while the other plate was poured with sterilized 10 per cent milk subsequently inoculated with butter culture and the plates then incubated to permit mold development. *Oospora lactis* was observed in only 4, or 6.7 per cent, of the 60 plates exposed.

The procedure followed for the detection of *Oospora lactis* in the miscellaneous materials examined was as follows: Quart and pint bottles one-half filled with milk were steamed for 30 minutes, then cooled to room temperature and inoculated with butter culture; approximately 5 grams of material to be studied were stirred into a quart bottle and approximately 1 gram of the same material was stirred into a pint bottle and the capped bottles were left undisturbed at room temperature for 3 to 5 days for the molds to grow; the presence of *Oospora lactis* in the cream on the surface of the milk could usually be accurately detected by macroscopic observation but a microscopic examination of the mold growth was always made for final verification.

The number of samples of each material examined by this procedure and the per cent of the samples in which *Oospora lactis* was found were as follows: barn floor dirt 13, 84.6 per cent; bedding 10, 60 per cent; brushings from cow 30, 83.3 per cent; barley and wheat 21, 28.6 per cent; corn 21, 19.1 per cent; cottonseed meal 11, 18.2 per cent; grain mixtures 37, 54.1 per cent; oats 20, 15 per cent; soybean meal 15, 6.7 per cent; alfalfa 15, 13.3 per cent; beet pulp 10, 20 per cent; lespedeza 33, 39.4 per cent; clover and timothy hay 15, 13.3 per cent; silage 43, 62.8 per cent; soybean hay 10, 10 per cent; straw 23, 17.4 per cent; fresh cow feces 91, 82.4 per cent; fresh horse feces 16, 87.5 per cent; barnyard soil 45, 75.6 per cent; field and garden soil 42, 28.6 per cent.

Thirty samples of fresh cow feces plated on acidified potato-dextrose agar gave *Oospora lactis* counts of less than 1 per gm. in 2 samples and over 10,000 per gm. in 5 samples with a maximum count of 34,000 per gm.

**M8. The Mold Mycelia Count as an Index of Quality of Butter.** P. R. ELLIKER AND B. E. HORRALL, *Purdue University Agricultural Experiment Station, West Lafayette, Ind.*

This study was based on results of mold mycelia counts and scores (organoleptic grade) on 1,385 commercial butter samples obtained monthly over a three-year period from Indiana and surrounding region.

No 92 score butter was illegal (mold mycelia counts over 60 per cent) in any of the four seasons of the year. During the winter period practically

no 89 or 90 score and about 20 per cent of the cooking grade butter was illegal. The spring period was similar except that some increase in illegal butters occurred in the 89 score group. In the summer period about 30 per cent of the 90, 60 per cent of the 89 and 75 per cent of the cooking grade butter was illegal. In the fall period about 11 per cent of the 90 score, 18 per cent 89 score and about 73 per cent of the cooking grade butter was illegal. According to organoleptic tests, actual quality of butter remained reasonably constant throughout the year with slightly more poor quality butter in the fall months, but mold content of butter soared to high levels in the summer and then gradually receded to a low level with colder weather. By mold mycelia standards over the entire period studied, about 50 per cent of all cooking grade butter, about 25 per cent of 89 score (commercial number 2 grade), about 10 per cent of 90 score and none of the 92 score butter would have been considered illegal.

Results indicate that organoleptic grade of cream and butter should be emphasized above other available quality tests if actual quality of commercial butter is to be improved in the area studied.

**M9. An *Aerobacter* Species in Whey Cream as a Cause of a Medicinal Flavor Encountered in Butter.** T. J. CLAYDON, *University of Arkansas, Fayetteville, Ark.*

During a two-year period at the University of Arkansas Creamery, a number of churnings of commercial butter were encountered that exhibited a definite medicinal flavor. The defect varied in intensity from slight to very disagreeable. Whey cream, occasionally used for a portion of the fat, was found to be the source of the flavor. While not all whey cream was affected, some lots showed the typical medicinal flavor encountered in the butter. The odor was also suggestive of the defect. The condition was observed at various times in whey cream from three separate cheese plants. Inspection of one of the plants failed to indicate the cause. Fly spray and chlorine compounds did not appear to be involved, although laboratory tests suggested the latter as a possibility.

During this time an outbreak of a medicinal flavor in market milk was encountered and traced to *Aerobacter aerogenes*. Laboratory cheese-making trials showed that, under certain conditions, this organism would produce the medicinal flavor and odor in whey. The defect was more intense in the whey cream and resulting butter.

Although first attempts to isolate *A. aerogenes* from slightly defective whey cream were unsuccessful, examinations of later shipments yielded considerable numbers of organisms of the *Aerobacter* group. This organism inoculated into milk set for cheese-making, frequently produced a medicinal flavor and odor in whey after the latter was held several hours at room temperature. Increased development of acid masked the defect. Butter made from the whey cream was characterized by the medicinal defect.



Preliminary studies indicated the organism to be *A. aerogenes*, which is common in raw milk and to a considerable extent in pasteurized cheese. Possible phenol production by the organism, under some conditions, might account for the medicinal flavor.

**M10. Some Chemical and Physical Properties of Washing Powders.**

P. S. LUCAS, *Michigan State College, East Lansing, Mich.*

In the following report 13 commercial washing powders, one acid cleaner, three single chemical detergents, and two wetting agents were checked for some of their chemical properties as related to bottle and can washing adaptability. The cost of bottle washing economy in terms of water and alkali required under varying conditions was calculated.

In total alkalinity, active caustic strength, and soda ash content these cleaners varied from neutral to high values. Several had high buffer values as determined by pH determinations made after successive additions of standard acid, but the tendency of manufacturers compounding washing mixtures, except for bottle washing, has been to depend less upon alkalinity than upon reduction of surface tension through addition of wetting agents for cleansing value in their product.

The amounts of water and alkali required to wash a milk bottle depend upon the frequency with which the washing solution is changed, and whether the strength of the solution is maintained at a constant value. Of the two the water used is but slightly affected, the amount varying from 0.8 to 0.95 gallons of soft water per quart bottle equivalent washed. The amount of alkali used in soft water is greatly affected. In 26- and 33-day trials one pound alkali was sufficient for 222 and 333 bottles, respectively. In a 14-day trial, with one initial charge only, one pound washed but 189 quart bottles. In this trial total alkalinity decreased from 4.05 to 3.1 per cent and pH from 11.2 to 10.75. However, when the washing solution was changed only after 68 days one pound of alkali was sufficient to wash 476 bottles.

**M11. Factors Entering into the Testing of Sour Cream for Extraneous Matter.** KENNETH M. RENNER, *Texas Technological College, Lubbock, Tex.*

Recently a number of creameries located in the Southwestern section of the United States have found it desirable to make sediment tests on sour cream, using a four-ounce sample instead of the two-ounce sample formerly in general use throughout the country.

It was found that entirely too many of the four-ounce samples refused to filter completely when the methods of sediment testing commonly used for the two-ounce sample were applied to the larger size sample.

Various factors entering into the testing of sour cream for extraneous matter were studied in order to determine their effect on the ability to secure complete filtration of four-ounce samples of cream.

The following sediment tests, recently published by the American Butter Institute, were used :

Method 1—Soda Solution Method (Illinois Method).

Method 2—Dry Soda Method.

Method 3—Acid Method (HCL Method recommended by Prof. M. G. Pederson, Texas Technological College).

(HCL Method recommended by Prof. B. A. Horrall, Purdue University).

Method 4—Texas Tech. Method (Calgon-Diversey U,S,N,-Citric Acid) Recommended by Prof. K. M. Renner, Texas Technological College.

Method 5—Special Solution, designated by the Manufacturer as X-300.

The following factors were found to have a direct influence on the ability to secure complete filtration of four-ounce samples of sour cream in testing for extraneous matter :

1. The hardness of the water used in the test.
2. The percentage of butterfat in the cream.
3. The age of the cream.
4. The physical condition of the cream (curd content).
5. The method of adding the testing solutions to the cream.

No one method of testing four-ounce samples of cream for extraneous matter was found which would work one hundred per cent. A new method of filtering cream to determine the amount of extraneous matter in cream has been developed by the Department of Dairy Manufactures, Texas Technological College, which appears to be approximately 95 per cent effective under the conditions studied. Certain methods which had previously been successful in the filtering of two-ounce samples of cream would not work on four-ounce samples.

**M12. The Problem of Extraneous Matter in Cheddar Cheese.** WALTER V. PRICE AND RAYMOND MIERSCH, *University of Wisconsin, Madison, Wis.*

The test for extraneous matter in cheese provides a method of detecting the use of unclean milk and unsanitary factory conditions, and a means of demonstrating the necessity of strict sanitation. When milk is efficiently filtered the test becomes useless to check farm practices. A low-power microscope must be used to identify sediment of animal or insect origin.

Distribution of extraneous matter is fairly uniform in a single cheddar but from cheese to cheese from a single vat it is not uniform. Cheese from any source should be tested regularly and frequently as a control measure.

The test can be used systematically to improve cheese factory operations if the control chart system of inspecting the quality of a manufactured product is applied. By this method (which is described in principle by L. E. Simon, "An Engineer's Manual of Statistical Methods," John Wiley, 1941) 60 consecutive daily tests provide data to describe factory performance. The control chart provides a working guide based on factory performance and indicates variations in quality in time to correct errors *before* rejections are necessary. Specifications can be written with suitable statistical considerations in mind so that a quality improvement program can be initiated even though it is impractical as yet to set industrial standards or tolerances.

The justification of this plan is based on the conviction that it is unreasonable to expect perfect sediment tests in cheese. Observations and data indicate that where it is necessary the elimination of excessive amounts of extraneous matter from cheese is practical; the reduction of contamination with undesirable types of foreign matter in the factory is possible; and that costs of doing so should not be prohibitive.

**M14. Use of Conductivity Measurements for Detecting Neutralization of Buttermilk.** S. T. COULTER AND R. W. KUNKEL, *University of Minnesota, St. Paul, Minn.*

Large quantities of dry buttermilk are manufactured in central drying plants which purchase the buttermilk from outlying creameries. Many of these plants manufacture dry sweet cream buttermilk. Some rapid method of analysis which would detect the addition of neutralizer to the cream from which the buttermilk was churned should be of assistance.

The alkalinity of the ash has been used as an index of neutralization in dry buttermilk. Based on the analyses of 114 samples of dry buttermilk ranging in ash alkalinity from 78 to 524, the conductivity of the reconstituted buttermilk increases directly with increase in ash alkalinity. The conductivity measurements ranged from 53.3 to  $79.8 \times 10^{-4}$  mhos at 25° C. The correlation coefficient between the two sets of data was 0.94.

This test has been used with fluid buttermilk and appears to be a useful tool for grading buttermilk in central drying plants. A suitable correction must be made for dilution of the buttermilk.

**M13. Correlating Lactic Acid Determinations with Practical Milk Quality Tests in Grading Milk for Manufactured Dairy Products.** I. A. GOULD AND J. M. JENSEN, *Michigan State College, East Lansing, Mich.*

The lactic acid content of condensed and dried milk reflects the quality of the milk which is used in the manufacture of these products. However, since the direct application of lactic acid determinations to raw milk as a routine grading test is impractical, a study was conducted with the view of

correlating the lactic acid results with more practical milk quality tests. Trials were conducted in which raw milk from various sources was incubated either at 25° C. or 35° C. and examined at intervals for (a) lactic acid by the Hillig colorimetric method, (b) acid associated flavors, (c) titrable acidity, (d) direct microscopic clump counts, (e) methylene blue reduction. Acid associated flavors were usually detected at lactic acid increases of 2-5 mg./100 grams and pronounced acid-type flavors resulted when the lactic acid increase amounted to 10 mg./100 grams or more. Microscopic clump counts varied widely with specific increases in lactic acid although a general direct relationship existed. Of the samples with lactic acid increases of 1-2 mg./100 grams, 72.4 per cent of the samples possessed bacterial counts of less than 5 million. Acid associated flavors often resulted with bacterial counts of 2 million per ml. The original milk usually had bacterial counts below 50,000 per ml. Methylene blue reduction time was in general directly related to the lactic acid content of the milk but a production of 5 mg./100 grams or more was required before the reduction time was consistently under one hour. On the average, titrable acidities agreed closely with the lactic acid results but determinations on individual samples varied widely. Differences in the types and strains of the predominant bacteria in the milk may influence the relationship between the lactic acid content and these various practical quality tests.

**M15. An Improved Technique for the Determination of the Volatile Acidity of Cheese.** K. L. SMILEY AND A. C. DAHLBERG, *Cornell University, Ithaca, N. Y.*

A method was developed for the determination of volatile acids in cheese. Cheese was ground in a 10 per cent phosphoric acid solution and the resulting mush was extracted repeatedly with ether in the presence of magnesium sulfate. The fatty acids were liberated from the ether solution by repeated washings with 0.1 N NaOH. All traces of ether were evaporated from the combined alkaline washings on an electric hot plate. The washings were then acidified to pH 2.0 and distilled in the presence of  $\text{MgSO}_4$ . The distillate was filtered through paper to separate the soluble from the insoluble acids. The soluble acids were titrated directly, using phenolphthalein. Neutral alcohol was used to rinse the condenser and dissolve the insoluble acids on the filter paper. The alcoholic solution was then titrated and the sum of the two titrations equals the volatile acidity of the cheese.

An ordinary Kjeldahl distillation outfit without traps was used for a condenser. Since the volatile acids are quantitatively distilled in the presence of  $\text{MgSO}_4$ , a perfectly uniform apparatus is not necessary.

The method gave uniform results for all samples of cheese and the results agree very well with values obtained using the method of Hiscox, Harrison, and Wolf. It has two advantages over the latter method in that no special apparatus is necessary and the time required is greatly reduced.

**M16. A Study of Some of the Substances Absorbed on the Fat Globules of Milk.** ROBERT JENNESS, *University of Minnesota, St. Paul, Minn.*

This study was undertaken to ascertain whether large variations in proportions of protein, phospholipide, and phosphorus-free lipid such as were found in natural "membranes" by Rimpila and Palmer (1935) could be correlated with such natural factors as breed of cow, season, ration, or stage of lactation. The method consisted of precipitation of the "membrane" protein from washed cream with dioxan and determination of lipid phosphorus in either the dioxan or Roese-Gottlieb extracts. Ranges of 0.459–0.862 gm. protein and 8.9–16.4 mgm. lipid phosphorus per 100 gm. fat were found in creams that had been washed 6 times at 105–100° F. The levels were relatively constant for any given group of cows, being lower in Jersey than in Holstein creams. The ratio of protein/phospholipide varied from 1.8 to 2.3 in 13 creams, being 2.7 in the fourteenth. It was calculated that 3.4–4.9 µg. protein and 0.57–0.86 µg. lipid phosphorus were adsorbed per 100 cm.<sup>2</sup> of fat surface.

The distribution of protein and phospholipide between washed cream buttermilk and butter plasma was followed by precipitating the protein with dioxan and determining lipid phosphorus in the dioxan extract. The ratio of protein/phospholipide was 2.4 to 3.8 in 11 samples of buttermilk and 1.0 to 2.0 in the corresponding butter plasmas. A rather constant retention in the butter plasma of protein at a level of 0.21–0.30 gm. per 100 gm. fat was noted regardless of concentration of fat or of protein in the unchurned washed cream. The level of phospholipide retained by the butter plasma was not constant but tended to vary directly with the level in the washed cream.

The presence in "membrane" preparations of the high melting triglyceride fraction noted by Palmer and Wiese (1933) was confirmed. Fractions of nearly identical properties were crystallized from alcohol extracts of washed cream buttermilk, and butter plasma and from alcohol solutions of butterfat. This material constituted a greater percentage of the phosphorus-free extract of butter plasma than of that of buttermilk or of butterfat itself.

These findings appear to be best explained by considering that the "membrane" consists of a lecithoprotein complex with phospholipides oriented to the fat phase and protein to the aqueous phase. Churning evidently involves "erosion" of protein to a critical level, the complex being disrupted in such a manner as to release a comparatively protein-rich portion to the free buttermilk. Upon melting the butter, the butter plasma is enriched in the higher melting fat fraction probably as a result of a special affinity of the "membrane" for it.

**M17. Some Factors Affecting the Inversion of Sucrose.** T. R. FREEMAN  
AND E. L. FOUTS, *University of Florida, Gainesville, Fla.*

Discussions in the literature regarding the relative sweetness of invert sugar indicate widespread disagreement and confusion on this subject. It has been tacitly assumed in all theoretical discussions that acid hydrolysis of sucrose by boiling in a water solution produces equal quantities of dextrose and levulose. It is also generally assumed that the sucrose is completely hydrolyzed. Both of these assumptions are erroneous. The present study was undertaken to obtain some basic information which might help eliminate, or at least account for, some of the divergent views regarding the sweetening power of invert sugar.

The effect on the inversion of sucrose of the kind and amount of acid employed has been studied. Tartaric, citric, lactic, acetic, hydrochloric, sulfuric, and phosphoric acids were included. A series of sirups was prepared with each acid, varying the amount of acid in such manner as to obtain pH values within the approximate range of 3.5 to 1.5. The resulting invert sirups were analyzed quantitatively for dextrose and levulose, using the "iodine-oxidation" method described by Lathrop and Holmes (*Indus. and Engin. Chem., Analyt. Ed.*, **3**: 334-339. 1931).

It has been observed that, in general, the most complete inversion of the sucrose occurs within the pH range of 2.5-2.0. The optimum pH for each acid and the degree to which the sucrose was hydrolyzed may be indicated as follows: tartaric, pH 2.09, 96.0 per cent; citric, pH 2.15, 95.1 per cent; lactic, pH 2.23, 95.0 per cent; acetic, pH 2.20, 98.4 per cent; HCl, pH 2.63, 95.3 per cent;  $\text{H}_2\text{SO}_4$ , pH 2.43, 95.3 per cent;  $\text{H}_3\text{PO}_4$ , pH 1.85, 93.4 per cent.

The invert sugars thus produced contained 41.1 to 51.1 per cent levulose. The effect of kind and amount of acid on ratio of dextrose to levulose in the resulting invert sugar was inconsistent. The pH which produced the most favorable ratio of levulose to dextrose, and the per cent levulose in the invert sugar, are indicated as follows for each acid: tartaric, pH 3.20, 48.5 per cent; citric, pH 3.41, 50.0 per cent; lactic, pH 2.68, 48.3 per cent; acetic, pH 2.20, 50.8 per cent; HCl, pH 2.63, 48.4 per cent;  $\text{H}_2\text{SO}_4$ , pH 3.20, 51.1 per cent;  $\text{H}_3\text{PO}_4$ , pH 3.24, 48.6 per cent. With only two of the acids used (acetic and hydrochloric) was the best ratio of levulose to dextrose obtained at the same pH which resulted in the maximum production of invert sugar.

Tartaric, citric, lactic, hydrochloric, and sulfuric acids gave satisfactory inversion of the sucrose. Phosphoric acid is a somewhat less efficient catalyst than the other acids studied. Although acetic acid gave the highest yield of invert sugar, and a very favorable ratio of levulose to dextrose, its use is impractical because of the unpleasant odor imparted to the sirup.

Preliminary trials indicate that the proportion of sugar to water used in preparing invert sirup affects hydrolysis of the sucrose. The yield of invert sugar and the per cent levulose in the invert sugar both increase as

the water content of the unboiled sirup is increased progressively from 27.8 per cent to 39.8 per cent.

**M18. Study of the Peroxide Value of Stored Spray-Dried Whole Milk Powder.** HARRY PYENSON AND P. H. TRACY, *University of Illinois, Urbana, Ill.*, AND J. M. TRIMBLE, *Indiana Condensed Milk Company, Indianapolis, Ind.*

Approximately 180 samples of spray-dried whole milk powder stored at room temperature and varying in age from fresh to 21 months were analyzed for peroxide content, gas content and scored for flavor. The variables included antioxidant addition, packing temperature, spray chamber temperature, spray pressure, spray temperature, pre-heat temperature, packing temperature, homogenization pressure and temperature, copper addition, neutralization, time of packing, and gas packing.

There were cases where both fresh spray-dried whole milk powder and oxidized samples several months old had a low peroxide value. Other observations showed that some whole milk powder gave a peroxide value but lacked oxidized flavor. There was no correlation between peroxide content of plain-packed and nitrogen-packed whole milk powder and the extent of oxidized flavor development. Some of the whole milk powder studied, although lacking in peroxide content, produced an oxidized flavor.

With the exception of one set of four samples 18 months old, the highest peroxide value obtained on any of the 180 samples was 15.07 milliequivalents of peroxide per kilogram of milk powder. Since in general high peroxide values were not obtained it is concluded that side reactions take place as the peroxides form producing other compounds such as aldehydes, ketones and keto-hydroxy compounds. It was also noted that the peroxide values were low in most of those samples which showed little or no oxygen. The data indicate that it is possible, especially in the case of fresh whole milk powder, to obtain a reducing action resulting in a "negative" peroxide value.

On the basis of the results obtained with the Chapman and McFarlane (Canadian Journal of Research, B, 21: 133-139. 1943) method for the determination of peroxides in whole milk powder, it is concluded that peroxide value is not a satisfactory criterion of the palatability or keeping quality of spray-dried whole milk powder.

**M19. The Determination of Lipase from Milk Extracted with Acetone and Ether.** PHILIP L. KELLY, *University of Arkansas, Fayetteville, Ark.*

Dried defatted milk was obtained by extracting one volume of milk twice with acetone, once with equal parts of acetone and ether and twice with ether each time using five volumes of solvent. A soft white precipitate was obtained which will keep indefinitely. Since it contained only 5 or 6 per cent moisture the milk and substrate were used alone without preservative. Some

of the samples were immediately extracted with ether to serve as blanks, the remainder were incubated at 37° C., usually for 24 hours, before extraction. Alcohol was added to the extract which was titrated against aqueous N/10 sodium hydroxide using phenolphthalein as the indicator. A sufficient amount of substrate was added to completely moisten the milk or similar results were obtained by adding a 20 per cent solution of the substrate in ether, later allowing the ether to evaporate.

Samples of dried cream showed considerably higher lipase activity than did samples of whole or skimmed milk. When milk was held in a refrigerator 24 hours before drying the lipase activity increased from 20 to 80 per cent indicating that the age of the milk is an important factor in comparative studies.

Tributyrin, diacetin, triacetin, tricaproin, trimyristin, ethyl-oleate, tripalmitin and sterilized butter oil were used as substrates, both with normal milk and with milk which developed rancidity. Series were made up of from 4 to 20 samples. Results were expressed as the average increase in titration per gram of dried milk used in a 24-hour incubation period.

With tributyrin the data were obtained from 9 series with normal milk and 12 series from milk developing rancidity. The normal series showed titrations from 0.03 to 0.25 ml. The rancid series showed titrations from 0.30 to 1.40.

The three normal series with tricaproin produced titrations from 0.08 to 0.10 while the one series with milk developing rancidity gave a titration of 0.16.

The triacetin samples in three normal series varied from 0.15 to 0.26 while the three rancid series ranged from 0.36 to 0.69. There seemed to be no significant differences, nor any consistency in the results when diacetin was the substrate. This was also true when ethyl-oleate was the substrate. Although four series were carried out with trimyristin there was no evidence showing that this was hydrolyzed by milk lipase.

With tripalmitin two series with normal milk showed titration of 0.07, and two series with rancid milk titrations of 0.08 and 0.19.

When butter oil was the substrate, two series with normal milk gave values of 0.05 to 0.08 while the four using milk which developed rancidity showed titrations of 0.10 to 0.12.

The milk which showed development of rancidity indicated a selective hydrolysis with the short chain triglycerides hydrolyzed to a much greater extent than with the long chain triglycerides.

**M20. The Influence of Butter Cultures and of Butter Flavors on the Quality of Butter.** H. C. OLSON AND P. E. JOHNSON, *Oklahoma A. and M. College, Stillwater, Okla.*

Several series of churnings were made to compare the flavor and keeping quality of butter made with (1) butter flavors added to the butter, (2)



butter culture incorporated into the butter, (3) butter culture added to the cream, and (4) control churning with nothing added to either cream or butter. Samples of both salted and of unsalted butter from each churning were held at about 45° F. and at -5° F. Flavor score and diacetyl determinations were made on the samples at various intervals.

The use of either butter culture or of butter flavors improved the flavor of fresh butter. Butter culture was superior to the butter flavors in improving the fresh flavor of butter. Adding the butter culture directly to the butter resulted in a more pleasing fresh flavor than was obtained by adding the culture to the cream, although the average diacetyl content of the former was not as high. The diacetyl content of the butter made with culture added to the cream or with culture incorporated into the butter increased during the first week or two of storage and then decreased gradually throughout the remainder of the storage period at 45° F. The increase was much greater with the unsalted butter into which butter culture had been incorporated than with any of the other samples. The diacetyl content of the butter made with butter flavors added decreased during storage, the most rapid decrease occurring with the unsalted samples during the first week of storage. Considerable variation occurred among various commercial butter flavors in their ability to impart a desirable flavor and to retain their flavor in butter.

The butter made with culture added to the cream had better keeping qualities than any of the other lots.

**M21. Effect of Various Bacteria on Diacetyl Content and Flavor of Butter.** P. R. ELLIKER, *Purdue University Agricultural Experiment Station, West Lafayette, Ind.*

A modification of the Pien, Baisse, and Martin orthodiaminobenzidine hydrochloride method for diacetyl determination proved accurate, highly sensitive and rapid and consequently was employed throughout this investigation.

A wide variety of bacteria, including several different genera, were able to destroy diacetyl added to milk. *Streptococcus lactis* exerted no effect on diacetyl under the same conditions. A number of butter-spoilage organisms, including *Pseudomonas fluorescens*, *Pseudomonas fluorescens liquefaciens*, *Pseudomonas fragi*, *Pseudomonas putrefaciens*, *Pseudomonas nigri-faciens*, and some unidentified non-pigmented species of *Pseudomonas*, markedly reduced the diacetyl content of experimental butters under controlled conditions at 15.6° C. (60° F.). Again *Str. lactis* did not affect the diacetyl content under similar conditions. Almost all of the diacetyl destroyed by *Ps. putrefaciens* during 10 days of growth in butter stored at 15.6° C. could be recovered as acetyl-methylcarbinol and 2, 3-butylene glycol. About three-fourths of that recovered was present as acetyl-methylcarbinol and one-fourth as butylene glycol.

Commercial butters known to consistently carry large numbers of bacteria of the *Ps. putrefaciens* type rapidly lost diacetyl and aroma during keeping-quality tests.

Results indicated that the factor responsible for inhibition of *Pseudomonas* species by butter starters in butter was *Str. lactis* or related bacteria rather than diacetyl.

**M22. The Effect of Storage Temperature on the Keeping Quality of Butter.** C. N. STARK, J. J. R. CAMPBELL, AND E. S. GUTHRIE, *Cornell University, Ithaca, N. Y.*

Reports from this laboratory and unpublished data, as well as practical plant experience, have shown that the more important butter-spoilage factors are: (1) natural milk enzymes; (2) harmful bacteria; (3) salt; and (4) acid. The best-keeping butter is made from high quality, sweet cream which has been pasteurized at 165° F. for 30 minutes (or an equivalent heat treatment); it is unsalted and not recontaminated with harmful bacteria during the buttermaking process. If several of the above-mentioned spoilage factors are present, their combined effect as well as the effect of each of these factors upon the other, make more difficult the correct evaluation of the part played by each of these factors.

Much of the present excellent keeping quality of butter is made possible because the butter is usually stored in a frozen condition, where microorganisms cannot grow and chemical spoilage rates are retarded. These experiments, the results of which are being reported here, were conducted to obtain more information on the significance of storage temperatures upon the keeping quality of butter.

It was assumed that commercial butter should be stored at or below freezing temperature. Many tests were made but the importance of storage of butter at or below 0° F. was definitely shown. For example, similar butters (sweet cream, pasteurized at 165° F. for 30 minutes, unsalted) stored at 0° F. and 32° F. respectively had the following scores: fresh, 95 and 95; stored 6 months, 93 and 93; stored 8 months, 93 and 91; stored one year, 93 and 83. The absence of appreciable numbers of microorganisms in the butter stored at 32° F. shows that microorganisms were not responsible for the spoilage of the butter stored at 32° F.

A long series of tests made in the Cornell Laboratories has shown such butter stored for as long as 8 years at 0° F. to score 92. These findings confirm the practice of 0° F. storage temperatures for commercial butter.

**M23. Butter Studies: Knaysi Method vs. Titration of Fatty Acids in Butter.** E. S. GUTHRIE, *Cornell University, Ithaca, N. Y.*

Neither method can replace judging. Of the 378 samples the Knaysi reading correlated more closely with the judging in 20 cases than did the

titration procedure. And it was evident in 22 cases that the titration procedure was closer to the judging than the Knaysi method. In 8 cases there was poor correlation between both methods and judging. These methods, therefore, correlate closely with each other and both agree with judging in the great majority of cases.

*Difference in score of Interior and surface.* The interior score of 378 samples of butter purchased from the open market was 91 with a range from 87-93, whereas the surface score was about 87 with a range from 83-92.

**M24. Action of Lipase from Various Sources on the Fat of Cheddar Cheese.** F. J. BABEL, *Iowa Agricultural Experiment Station, Ames, Ia.*

The fat acidities of cheddar cheese made from pasteurized milk, without addition of lipase, increased at a rather regular rate during the ripening period. The results did not indicate a more rapid breakdown of the fat late in the ripening. The variations encountered in the fat acidities with the different lots of cheese could not be correlated with the pH of the cheese, the acidity of the whey at milling, or the moisture or fat contents of the cheese.

Cheese made with small amounts of rennet paste showed definite increases in fat acidities over those of the control cheese. The cheese either did not develop a rancid flavor or the rancid flavor disappeared during the ripening. The addition of small amounts of rennet paste appeared to overcome the sour or acid flavor frequently encountered in pasteurized milk cheese. Larger amounts of rennet paste resulted in greater increases in the fat acidities although variations were encountered. With the larger amounts, the cheese developed a rancid flavor and this was usually evident throughout the ripening; however, in some cases the rancid flavor appeared to decrease in intensity as the cheese became older.

Cheese made with small amounts of mulberry juice added to the milk either did not increase in fat acidity or only slightly increased. The small amounts did not produce a rancid flavor in the cheese and the cheese were very similar to the control cheese. Larger amounts of mulberry juice resulted in considerably greater increases in fat acidities; however, variations were encountered with different lots of cheese and different lots of mulberries. The rancid flavor of the cheese made with mulberry juice did not appear to decrease in intensity as the cheese became older. Unclean and rancid flavors were frequently encountered. In practically all cases, the cheese made with added mulberry juice was considered inferior to the control cheese.

**M25. Combining Lactic and Bulgaricus Fermentation in Cheese-Making to Prevent Gas Formation in Cheese-Ripening.** N. S. GOLDING, *Washington Agricultural Experiment Station, Pullman, Wash.*

For the successful vacuum canning of cheese, it is essential that the

freshly canned cheese do not produce sufficient gas during the ripening process to bulge the cans. We have found that when the starter rapidly produced acid during the making process, subsequent gas production during ripening is slight. In contrast, when the starter failed to produce acid actively during making, sufficient gas to bulge the ends of the cans was produced at ripening temperatures from 40° F. to 50° F. Of 270 cans of cheese made by the cheddar process, 13.5 per cent produced sufficient gas to be at atmospheric pressure or higher when mature. The more complete the failure of the lactic fermentation and the higher the temperature of ripening, the more rapidly gas formation occurred during ripening. Also, it was found that different commercial lactic starters for cheese-making changed materially the rate of production of gas when the cheeses were ripened under the same conditions.

*Lactobacillus bulgaricus*, which readily grows in milk and rapidly utilizes lactose with the production of a high degree of acid, but with no gas, was obtained as a commercial starter. This starter and a commercial lactic starter were used together in the manufacture of cheese having a setting-to-milling time of from 4.5 to 5 hours and an acidity at milling of 0.5 per cent. In all cases, the addition of the *bulgaricus* starter resulted in less gas being produced in the mature can of cheese; the greater the proportion of *bulgaricus* starter used, the less the amount of gas produced. The mature cheese made with the combined starters do not have a typical cheddar flavor, but possess a clean, sweet flavor which is acceptable to many people. The texture was that of a Cheshire cheese and the color was usually uneven. The mature cheese withstands adverse storage conditions of 55° and 65° F., frequently encountered in retail stores, as little or no gas was produced at these temperatures during a period of 50 days. Experiments now under way include methods for determining to what extent the lactic and *bulgaricus* starters develop during the making process as follows:

- a. The amount of acidity produced in the milk by each starter by holding it at the cheese-making temperatures in sterile containers and determining the per cent of acidity at dipping and milling.
- b. Dilution counts in milk incubated at 21° C. and 37° C.
- c. Plate counts on tryptone glucose extract agar incubated at 20° C., and Baltimore Biological Laboratories anaerobic agar incubated at 37° C.
- d. Microscopic counts by a modification of the Breed method. Cheese of 2041 grams each were canned the day after manufacturing in No. 10, 5" cans and ripened at three different temperatures; namely, 45° F., 55° F., and 65° F.

The results thus far obtained over a period of 50 days (a period which has previously represented more than half the gas formed during ripening) show:

- a. With lactic starter and little or no *bulgaricus* starter, the average drop in vacuum was 10,  $7\frac{1}{2}$  and 6 inches of mercury for temperatures of 65, 55, and 45° F., respectively.
- b. With lactic and *bulgaricus* starters added in equal proportion, the average drop in vacuum was 3, 3, and  $2\frac{3}{4}$  inches of mercury for temperatures of 65, 55, and 45° F., respectively.
- c. With *bulgaricus* starter only, the average drop in vacuum was  $\frac{3}{4}$ ,  $1\frac{1}{4}$  and  $1\frac{1}{2}$  inches of mercury for temperatures of 65, 55, and 45° F., respectively. Thus far, these results are in agreement with our previous work.

**M27. Effect of Heat Treatments of Milk on Quality and Ripening of Cheddar Cheese.** A. O. CALL AND W. V. PRICE, *University of Wisconsin, Madison, Wis.*

The Cheddar cheese for these experiments was made between October, 1942, and June, 1943. Each experimental group of cheese included four lots made from identical milk; one lot of cheese was always made from raw milk, one from milk pasteurized at 160° F. for 15.6 seconds and the other two lots from portions of the milk which had been subjected to some other heat treatments. The Cherry-Burrell Fine-line, Continuous Pasteurizer was used for all short-time heat treatments. The cheese was scored at 1, 3, 6 and 12 months by several judges and was analyzed for water-soluble nitrogen and amino nitrogen contents at the ages of 3, 6 and 12 months.

Since the final analyses have just been completed it is possible to present only a preliminary report on the results. The data confirm the conclusions of former studies by many workers who have shown the value of proper pasteurization for improving the quality and uniformity of Cheddar cheese. Heat treatments of milk which exceed or fail to meet pasteurizing requirements produce cheese which is intermediate in quality between raw-milk and properly-pasteurized-milk cheese.

The amounts of nitrogen in the cheese in the water-soluble and amino nitrogen forms vary with the severity of the heat treatment and with the age of the cheese. These values are more uniform during the curing of pasteurized-milk cheese. There seems to be a tendency for these values to increase as the quality of the raw-milk cheese decreases but they do not seem to be so closely related to the quality of cheese made from heated milk.

In these experiments heat treatments which approximated true pasteurization produced the effects of usual pasteurizing heat treatments.

**M28. Relation of Corn and Alfalfa Silage to the Quality of Cheese and Its Carotene and Vitamin-A Content.** W. V. PRICE, K. HIGUCHI, AND W. H. PETERSON, *University of Wisconsin, Madison, Wis.*

The milks from three groups of cows were made simultaneously into

cheese. The rations of these groups differed only in the type of silage which each received; one group (I) was fed corn silage, the second (II) half corn and half alfalfa silage and the third (III) alfalfa silage.\* The complete milkings in the evening and morning were combined for these cheese-making experiments. All lots of milk contained less than 15,000 organisms per ml. when placed in the cheese vats. The cheese was made on eight different days during the months of January and February, 1944. All lots of cheese were paraffined, cured at 40° F., and scored at 3 months of age by three judges.

The flavor scores for lots I, II, and III averaged 37.5, 37.9 and 38.0, respectively; listed in the same order, body and texture scores averaged 28.0, 28.4 and 28.3; while total scores averaged 90.4, 91.2 and 91.3. Such small differences in quality in favor of the milk from alfalfa-silage-fed cows are noteworthy because they tend to refute, at least for cheddar cheese, the notion of some practical cheesemakers that alfalfa silage cannot be fed without causing defective cheese. No differences were observed in manufacturing procedures for these three lots of milk.

All whey was saved from each vat; to each lot of whey was added one-third of the press drippings from all three lots of cheese and this mixture was used for measuring carotene and vitamin-A distribution in the making process. Cheese was sampled for analysis as it was removed from the press, again at 8 weeks and finally at 12 weeks after making.

The carotene and vitamin-A content of each lot of cheese paralleled the values found for the milk from which it was made. There was no destruction of carotene and vitamin A during a 12-week curing period. At the end of this time the micrograms of carotene per gram of cheese for lots I, II and III were 1.58, 1.88 and 1.85, respectively. The corresponding figures for vitamin A were 1.84, 1.98 and 2.28. About 85 per cent of the carotene and vitamin A in the milk was recovered in the cheese, 7 per cent was left in the whey and 8 per cent was not accounted for.

**M29. Two Years' Experience in Deaerating Milk.** E. S. GUTHRIE, *Cornell University, Ithaca, N. Y.*

Taking April, 1943, as a typical month for intensive oxidized flavors in milk, the scores were as follows: fresh milk, not deaerated, 40; deaerated milk that was put in the bottle by the in-bottom filling procedure, not under vacuum, 39 on the 7th day; deaerated milk which was conveyed to the bottle by a commercial filler, not under vacuum, 36 on the 7th day; and the control sample that was not deaerated, 31 on the 7th day.

In April, 1943, the vitamin C dropped from 18.24 milligrams per liter of the original milk to 16.89 in the in-bottom sample that was held 7 days.

\* J. J. Stefaniak, I. W. Rupel, and W. H. Peterson. "Effect of feeding corn and alfalfa silages on the fat- and water-soluble vitamins of winter milk." Proceedings of the 39th Annual Meeting, American Dairy Science Association, Columbus, Ohio, June, 1944.

The vitamin C in the commercially filled bottles was 15.32 milligrams on the 7th day, and the control sample that was not deaerated was down to 7.74 on the 7th day.

During the period of over 2 years of eliminating almost all of the oxygen from the milk on a commercial basis, the consumers have not complained about the oxidized flavors; whereas, previous to the installation of the deaerating equipment, these flavors were the cause of much consumer criticism.

**M30. The Relationship of the Individuality of the Cow to the Production of Rancid Milk.** W. A. KRIENKE, *Oklahoma A. and M. College, Stillwater, Okla.*

Individual samples of milk were taken from each producing cow in the college dairy herd at two-week intervals during 1942 and at monthly intervals during 1943. All samples were cooled in ice water immediately after milking and held at about 38° F. for one day and for three days. The samples were then examined organoleptically for the rancid flavor and odor.

A high percentage of the cows were found to produce rancid milk during this period; some were very persistent in producing it while others produced it occasionally and at irregular intervals. Several cows produced the defect at the beginning of their lactation periods while others produced it only at the end. Rancid milk was produced by some cows at the beginning and at the end of their lactation periods with an intermediate interval during which the defect was not present in the milk. A few cows which produced the defect during the second lactation period of this study had not produced it during the initial lactation period. Several cows produced the defect during the initial lactation period but not during the succeeding one, even though some of them had been persistent producers of rancid milk. A number of cows produced the defect during successive lactation periods.

In general the rancid flavor and odor was most pronounced in milk from the persistent producers of the defect.

**M31. Milk as a Frozen Food (A Preliminary Report on Technical Aspects).** F. J. DOAN AND J. G. LEEDER, *Pennsylvania Agricultural Experiment Station, State College, Pa.*

Experiments have demonstrated the possibility of manufacturing a frozen concentrated form of milk which may be distributed to the consumer in a manner similar to that used for other types of frozen food.

The milk is pasteurized at 180° F. for 15 minutes, then it is concentrated at a ratio of approximately three to one. After condensing, the hot milk is homogenized at a pressure of at least 3,000 lb. and is then cooled to 40° F. to await initial freezing. Initial freezing is accomplished in either a batch or continuous type of ice cream freezer, the continuous being prefer-

ble for several reasons. A minimum amount of air incorporation during reeizing is desirable.

When frozen, the milk may be packaged in ice cream containers. The packaged milk is placed in a hardening room for rapid hardening before being stored. The temperature of frozen storage bears a definite relationship to the properties of the protein in the reconstituted product. Temperatures above  $-10^{\circ}$  F. are unsatisfactory from this standpoint.

The most satisfactory method of reconstitution is to place the frozen block of milk into the proper volume of hot water ( $180^{\circ}$  F.) and to allow it to melt undisturbed. After melting the milk should be stirred to make it homogeneous.

The flavor of reconstituted frozen concentrated milk is little different from fresh fluid, pasteurized, homogenized milk. When pasteurized at the recommended temperature, the flavor is somewhat more "cooked" than ordinary milk but this flavor gradually disappears during storage. The objectionable flavors developed during long holding in storage are "stale" and oxidized. The former does not become objectionable until long after the milk would be used under commercial conditions. The latter flavor may develop unless precautions are taken to eliminate copper contamination.

**M32. The Utilization of Skim Milk in Ice Cream Mix.** W. S. ARBUCKLE, C. N. SHEPARDSON, AND H. M. WALLING, *Texas Agricultural Experiment Station, College Station, Tex.*

This paper presents information upon the preparation and use of a concentrated skim milk product for the manufacture of ice cream. The method which proved most successful consisted of coagulating skim milk at a temperature of  $94$  to  $96^{\circ}$  F. with 10 per cent hydrochloric acid, draining the whey at an acidity of 0.45 to 0.48 per cent, disintegrating the curd by passing it through the screen bottom of a drain rack and then redissolving the curd by use of 5 pounds of sodium bicarbonate per 100 pounds of casein and by heating to  $150^{\circ}$  F. for 30 minutes. The product was standardized to 16 per cent solids content by the addition of skim milk according to the Pearson Square method or to a Baumé reading of 7.2 to  $60^{\circ}$  F. The quality and composition control was based upon the use of the acidity tester, a casein test and the hydrometer. The preparation was simplified by completing the entire process by the use of a cheese vat as the major piece of equipment. Disintegrating and heating the curd brought about more rapid dissolving. The heating period reduced the bacterial count of the finished product to fifty thousand or less. The use of skim milk to standardize the finished product to 16 per cent produced a product that was easier to handle and also offered a means of conserving additional non-fat milk solids. An analysis showed that the approximate composition of the finished product was as follows: 16 per cent total solids, 84 per cent water, 13.8 per cent protein, 2 per



cent lactose and 1.5 per cent ash. The cost of manufacture excluding labor was approximately one and one-half cents per pound solids.

The product prepared in this manner was used to supply 10 to 50 per cent of the serum solids content of various ice cream mixes. The data indicate that 37.5 per cent of the serum solids can be supplied by the concentrated skim milk in a 10 per cent fat, 8 per cent serum solids ice cream containing butter as a source of fat. When 40 per cent cream was used as a source of fat, the amount of serum solids that could be furnished by the concentrated skim milk in a 10 per cent fat, 8 per cent serum solids, a 12 per cent fat, 9.6 per cent serum solids and a 12 per cent fat, 11 per cent serum solids mix was 10, 20 and 50 per cent, respectively.

**M33. Factors Affecting the Oxygen Content of the Gaseous Phase of Packaged Whole Milk Powder.** J. H. HETRICK AND P. H. TRACY, *University of Illinois, Urbana, Ill.*

Particles of spray-dried milk powder contain occluded oxygen which is not removed by the ordinary process of gas packing. Packing milk powder in an atmosphere extremely low in oxygen content, therefore, becomes a problem of control of occluded oxygen as well as one of proper technique in packing. The oxygen entrapped in the particle slowly diffuses into the headspace until equilibrium is reached with respect to oxygen concentration inside and outside the particles. By means of the values for oxygen concentration in the headspace gas immediately after packing and after diffusion was complete, the relative volume of oxygen entrapped per gram of powder was calculated for the milk powders reported in these experiments.

Difference in manufacturing procedure accounted for some differences in the oxygen content of the powder when a standard procedure for gas packing was used.

When the method of spraying was varied, the powder dried using a small orifice and high pressure contained a higher oxygen content per gram than that dried using a larger orifice and lower pressure.

When the method of spraying was fixed and the total solids of the condensed varied from 31–38–45 per cent total solids, the powder made from the condensed having the highest total solids showed the lowest oxygen content per gram.

Powder packed from the drier at a temperature of approximately 120–125° F. showed less oxygen content per gram than powder cooled to 60° F. before packing.

Powder packed immediately after drying at 120–125° F. showed a lower oxygen content per gram than powder aged 18–24 hours before packing.

The addition of 200 parts per million of sodium ascorbate to the condensed before drying resulted in a lower oxygen content per gram of powder over the control with no treatment.

When air was incorporated in the condensed milk before drying by agitation while spraying, an increase in oxygen content per gram of powder was noted over the control in which special effort was used to keep the amount of air incorporated at a minimum.

It would appear from the data that the amount of oxygen entrapped per gram of powder was in part a function of the oxygen dissolved in milk before drying.

The results of a study of some of the factors in technique of gas packing showed that when evacuating to an absolute pressure of 3 mm., no appreciable lowering of oxygen content was noted by holding at this pressure for longer than 10 minutes up to 40 minutes. When powder was packed warm, an increase in oxygen content was observed as the length of time elapsing on soldering was increased. Under the conditions of the experiments reported, it appears that if proper methods are used in preparing the milk for drying, evacuation to an absolute pressure less than 12 mm. was necessary to meet the 3 per cent by volume maximum oxygen requirement as specified by the Quartermaster Corps.

It seemed that in control of oxygen content, the factors in the technique of packing showed more variation than factors in processing and that multiple gas packing was the most effective method for reducing the oxygen content to a minimum.

**M34. The Keeping Quality of Commercial Dried Whole Milk Packaged in Air and Nitrogen.** G. R. GREENBANK, P. A. WRIGHT, AND E. F. DEYSHER, *Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.*

A study was made of the effect of inert gas on the keeping quality of commercially manufactured and packed dried whole milk. The general conclusions are based on a study of 1,500 1-pound tins of dried whole milk, and specific data are presented on 350 tins of the spray-dried product. Packaging in inert gas, with approximately 3 to 4 per cent of oxygen in the container, may be conservatively assumed to increase the keeping quality at least 100 per cent, compared with packaging in air. A comparison of accelerated and normal aging was made. Flavor and peroxide values were used as indices of the keeping quality, and are discussed.

**M35. Further Observations Dealing with the Behavior of Ascorbic Acid in Evaporated Milk.** D. V. JOSEPHSON AND F. J. DOAN, *Pennsylvania Agricultural Experiment Station, State College, Pa.*

A comprehensive survey of the ascorbic acid content of commercial evaporated milks was conducted and reported previously as well as a study of fortification of evaporated milk with this vitamin.

Recent work dealing with fortification at higher levels shows that additions up to 100 mg. per liter (reconstituted) are practical. However, at this level there is some evidence of protein de-stabilization or coagulation during sterilization. This instability characteristic can be controlled by the use of the commonly used stabilizing salts.

Studies with the use of the stabilizing salts show that, on a molar basis, sodium citrate is most effective in preventing coagulation, with disodium phosphate and sodium bicarbonate somewhat less effective.

High temperatures of storage result in greater loss of ascorbic acid than are experienced at lower temperatures.

It has been found that the copper content of the milk is gradually depleted as the storage period progresses.

When infant formulas are prepared from fortified evaporated milk and stored at home refrigerator temperatures, the loss of ascorbic acid is surprisingly low during a 72-hour period. Boiled formulas show somewhat higher losses than unboiled.

**M36. A Comparison of the Different Types of Sweetening Agents as Preservatives of Condensed Milk.** W. A. HOSKISSON, P. H. TRACY, AND M. J. PRUCHA,\* *University of Illinois, Urbana, Ill.*

Six sweetening agents, sucrose, dextrose, Frodex, Sweetose, Confectioners' corn sirup and invert sirup were compared to determine their preserving power in both sweetened condensed skimmilk and sugar-broth solutions.

Six microorganisms, *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus pikowskyi*, a heat resistant organism isolated from evaporated milk, *Saccharomyces cerevisiae*, and a yeast isolated from corn sirup, were used as test organisms to determine the preserving properties of the six sweetening agents.

Both the sugar-broth solutions and the sweetened condensed skimmilk samples were stored at room temperature for the entire period of the study.

The sugar-broth and sweetened condensed skimmilk were plated at the beginning and periodically during the study to determine the number of organisms surviving in the samples. The bacterial plates were incubated at 32° C. for three days and the yeast plates at approximately 24° C. for 5 days.

From the sugar-broth experiments it was found that those sweetening agents such as invert sirup, Sweetose and the dextrose-sucrose combination which are high in monosaccharide content were more effective preserving agents than those low in monosaccharide contents such as Frodex, Confectioners' corn sirup or a disaccharide such as sucrose.

\* Emeritus.

The preserving action of the sugars in the broth solutions varied with the organism concerned. The yeast isolated from corn sirup was not inactivated in a total sugar-water content of 68 per cent when using sucrose, Frodex or Confectioners' corn sirup, while with the dextrose-sucrose combination 66 per cent sugar-water solution was sufficient to stop its growth. The growth of the yeast was stopped by a 62 per cent solution of invert sirup. The bacteria were generally inactivated at a lesser concentration of the sweetening agents than this yeast. *Saccharomyces cerevisiae* was inactivated by all the sweetening agents in a 60 per cent sugar-broth solution.

The preserving action of the six sweetening agents was studied in sweetened condensed skimmilk containing 42 per cent sugar, 50 per cent of which was sucrose and 50 per cent supplied by one of the other sweetening agents. In the sweetened condensed skimmilk, as in the sugar-broth solutions, those sweetening agents high in monosaccharide content were, in general, better preserving agents than those low in monosaccharide content or disaccharide such as sucrose.

In using the Frodex or Confectioners' corn sirup, it was found that these products had a tendency to cause thickening of the milk if used in quantities as high as 50 per cent of the sugar needed in the condensed milk. It is recommended that the replacement be limited to 25 to 33.33 per cent.

In using invert sirup in sweetened condensed skimmilk, it was found that this product, though excellent as a preserving agent, had a tendency to cause a brown color to develop in the product as it came from the vacuum pan.

In general, sweetening agents high in monosaccharide content, such as invert sirup, Sweetose, and Cerelose, are more efficient preserving agents than those low in monosaccharide content such as Frodex or Confectioners' corn sirup or a disaccharide such as sucrose.

As the concentration of the sweetening agent is increased its preserving power is also increased.

Sweetose is a more efficient preserving agent than either Frodex or Confectioners' corn sirup. Its greater preserving power is evidently due to its higher total sugar and monosaccharide content.

The preserving action of any of the sweetening agents used varies with the organism concerned.

Sweetened condensed milk containing 28 per cent milk solids and 42 per cent sugar, 50 per cent of which is sucrose and 50 per cent of either invert sirup, Sweetose or Cerelose compared to sweetened condensed milk made with 100 per cent sucrose, the former products can be safely stored at room temperature without danger of spoilage by most bacteria or yeast.

Until more is known about the nature and structure of cellplasma membranes and mechanisms by which substances in solution pass through them, it is not possible to state how sweetening agents exert a preserving action on microorganisms.

**M37. Iron Content of Evaporated Milk as Related to Greenish-Black Discoloration in Mixtures of Coffee and Evaporated Milk.** W. C. COLE AND N. P. TARASSUK, *University of California, Davis, Calif.*

Evaporated milk is often added to coffee as a means of modifying the color and flavor. Under certain conditions a dark-greenish or greenish-black discoloration occurs as soon as the milk is added. Although this phenomenon is fairly common, apparently neither the evaporated-milk industry nor dairy research workers have seriously studied the problem.

Observations with common commercial brands of evaporated milk revealed that this discoloration did not appear when milk from freshly opened cans was added to coffee, but that it sometimes developed when milk was stored in the opened can. The discoloration occurred more often if only a little milk was left in the can. Once it had appeared, it became more intense as the time of holding was prolonged.

The iron content of evaporated milk has been shown to increase rapidly after cans of milk are opened, especially if they are only a fourth or less full. This iron reacts with the tannin or tannin-like substances in coffee to produce the color change. Phosphates and citrates added to evaporated milk may modify the results, but if used within the limits of commercial practice will not significantly influence the tendency toward this discoloration. The reaction of the coffee-and-milk mixture likewise influences the results, since the extent of discoloration as well as the color itself depend upon the pH of the system.

Evaporated milk that has been stored in glass or porcelain after the can is opened will not cause the greenish-black discoloration when added to coffee. If the milk must be held more than 4 or 5 days after opening, it may well be poured into a suitable container.

**M38. Can we Hold Our Wartime Marketing Gains in Postwar Adjustments?** C. G. MCBRIDE, *Ohio State University, Columbus, Ohio.*

Wartime savings in milk marketing have been in reduction of use of trucks, tires, and gasoline in country assembling and city distribution, the considerable saving of man power due to every-other-day delivery and some reduction in number of products and packages carried. These savings are shown by research in many states on country hauling and New Jersey, New York, and Ohio on city distribution.

Sherman, of the Ohio Agricultural Experiment Station, estimates a possible saving of 20 per cent in country hauling. Not all of this has yet been made. Spencer, of Cornell University, has shown that costs of distribution have been lowered in cities in New York and New Jersey by reduction in number of deliveries. McBride and Keith in Ohio found reduction in mileage as high as 40 per cent and in drivers employed as high as 21 per cent.

Whether these marketing gains can be held depends upon several factors, including the attitudes of producers, dealers, labor, and the consumer. Ohio research indicates a high degree of consumer acceptance and cooperation in the matter of fewer deliveries and a narrower range of products. It was evident from the comments of 1,628 housewives interviewed that by many it was considered a patriotic duty to take such minor inconveniences as a part of wartime sacrifice. They were also favorably impressed by the fact that prices of dairy products had not gone up as fast as those of many other foods.

If wartime-gains are held in the postwar period it will be because of sound economic solutions of the following problems:

1. Will farmers be willing to continue with milk trucking as adjusted under the Office of Defense Transportation?
2. Will pressure be brought by labor unions to increase the number of jobs in milk delivery resulting in return to daily delivery and lighter loads?
3. Will dealers in a fierce competitive struggle again multiply the services and variety of products offered the housewife and thereby increase again the costs of distribution?
4. Will housewives continue their favorable attitude and be willing to hold the gains made or will they ask for a return of the old system even at greater cost.

It is difficult to get the answers to these questions in advance. A study just completed in Coshocton, Ohio, in which 587 housewives were interviewed, throws some light on present thinking on two points. They were asked if they would be satisfied with one cream of 30 per cent butterfat after the war. Only 187 of the 587 had bought cream the week of the survey. Of these, 157 said yes and 30 said no. The response with respect to every-other-day delivery was more conclusive. Four hundred and two took all or part of their milk by home delivery. Of these 327 expressed a willingness to continue on every-other-day delivery and 53 said they were not willing, and 22 did not commit themselves.

In the final adjustment much will depend upon the bargaining strength of the producers, dealers, and labor. If no one of these three groups have disproportionate power the chances are good that many of the marketing gains made under the stress of wartime regulation may be held. The extent to which the government will remain in the picture will depend upon how well the industry through cooperative efforts succeeds in holding the gains.

## THE TRAINING AND EMPLOYMENT OF MILK SANITARIANS: A COMMITTEE REPORT\*

H. F. JUDKINS, *Chairman*, F. H. HERZER, L. H. BURGWALD, P. H. TRACY,  
C. E. WYLIE, AND C. L. ROADHOUSE

*American Dairy Science Association*

### DEFINITION OF "MILK SANITARIAN"

After spending considerable time in the study of the duties of this committee, it occurred to the chairman that "milk sanitarian" should be defined. He therefore wrote to a number of milk control officials asking them to define "milk sanitarian." Several replies were received.

President C. A. Abele, of the International Association of Milk Sanitarians, has paraphrased Article II of their constitution to define a milk sanitarian as follows: "A milk sanitarian is one who is interested in the development of uniform and proper supervision and inspection of dairy farms, milk and milk products establishments, and milk and milk products; in the improvement in quality of dairy products and the technological development of dairy equipment and supplies; and in the dissemination of useful information regarding dairy sanitation, technology, inspection and administration." This definition is perhaps a little long, although quite fully descriptive.

The following definition from Mr. W. D. Tiedman, of the New York State Department of Health, is brief and yet very much to the point: "A milk sanitarian is a person making a profession of the application of sanitary and health measures to the production, processing and distribution of milk and milk products for the public benefit."

Mr. A. W. Fuchs, of the U. S. Public Health Service, also gives us a concise definition: "A milk sanitarian is a person who is qualified by technical training and experience to supervise the production, processing and distribution of an adequate supply of clean, safe, palatable milk and milk products in the interest of public health."

It is certainly gratifying to note in Mr. Fuchs' definition the statement which should probably be underlined several times, "in the interest of public health." Milk control agencies and officials should always be sure every regulation issued is directly related to public health. Sometimes they are related only to the economics of business operation.

### DUTIES, GENERAL QUALIFICATIONS, EDUCATION AND TRAINING

A letter was written to a considerable number of milk sanitarians and

\* Reprints of this report may be obtained by writing to R. B. Stoltz, Secretary-Treasurer of the American Dairy Science Association, Columbus, Ohio, previous to November 1, 1944.

heads of dairy departments to get their conception as to the duties, general qualifications, education and training of a milk sanitarian. The replies from these people are summarized as follows:

### *Duties*

1. Inspect dairy farms, receiving stations and processing plants.
2. Supervise design of plants and construction and remodelling of farm dairy buildings.
3. Approve design and test the operation of equipment.
4. Enforce health regulations, and administer licensing system.
5. Prevent spread of disease.
6. Check operations of water and sewage plants and the safety of water supplies.
7. Judge dairy products quality.
8. Inspect health of cows.
9. Make special investigations such as suspected milk-borne outbreaks.
10. Make chemical and bacteriological examinations.
11. Represent health department's case in court.
12. Recognize infectious disease hazards among employees.
13. Inspect dairy vehicles in city.
14. Determine temperature of milk products.
15. Collect samples.
16. Prepare and keep accurate reports.
17. Answer complaints and inquiries.
18. Discuss policies with Public Health Engineer.
19. Help train new inspectors.
20. Increase consumption of dairy products by building confidence in them.
21. Keep up-to-date by:
  - (a) Joining organizations that will increase knowledge.
  - (b) Attending short courses of instruction.
  - (c) Reading technical and trade publications.
  - (d) Trying out new ideas that seem practical.
22. Assist producers and operators in solving their sanitation problems.
23. Promotion of adoption of a satisfactory ordinance or amendments thereto.

### *General Qualifications*

A milk sanitarian must have "common sense," sound judgment and the makings of an administrator. He should be able and willing to teach others. He must be diplomatic, alert and practical. He must have a good personality, be able to sell his service and be interested in people. He must be able to gain the confidence of producer, distributor, and consumer, always of unquestioned integrity and never showing partiality. He must be in



good health, with energy and enthusiasm for his work. A keen interest in public health is an important qualification.

### *Education*

It is the opinion of the committee that the educational experiences of the student training for employment as a milk sanitarian should be somewhat broader than those provided by the usual dairy curriculum. Courses should be selected to provide a working knowledge in the following subjects:

- Feeding, breeding and management of dairy cattle;
- Veterinary science as related to the anatomy and diseases of dairy cattle and the relation of these diseases to a safe milk supply and the economics of production;
- Sanitary engineering—sanitary supervision of ground water supplies; water filtration and treatment; chlorination equipment and its use; domestic and creamery waste disposal; air conditioning; humidity control; air filtration; sanitary plumbing and drainage; vermin, rodent and fly control;
- Dairy products processing including sanitary quality of all ingredients used other than dairy products;
- Relation of milk to the public health emphasizing modes of infection, carriers, protective measures, medical examinations and procedures, epidemiology, diseases of man transmitted through milk;
- Food chemistry with necessary prerequisites;
- Dairy and pathogenic bacteriology with necessary prerequisites;
- Dairy engineering emphasizing refrigeration, heat transfers, temperature control, electric appliances and control, operating methods and maintenance of dairy machinery, sanitary design and construction of dairy equipment and dairy buildings, plan reading;
- Mathematics, physics and accounting;
- Federal, State and Municipal law enforcement;
- Costs and methods of production, transportation, processing and distributing milk and its products;
- Laboratory control and analysis of milk and milk products;
- Course should include six months to a year of "intern training" with selected health departments or leading processors of milk or milk products engaged in progressive quality improvement program;
- English, including public speaking;
- A course dealing with the appraisal of the U.S.P.H.S. Standard Ordinance and other ordinances to include the technique (with practice) of making farm and plant inspections;
- Business administration.

One health officer states in effect that large state and city health departments can employ specialists, and those with the best personalities and who

develop the best administrative ability are the ones likely to rise to the top positions. Thus it is pointed out that sanitary engineers with some dairy training may be expected to make the best plant equipment and plant process specialists. Veterinarians should make the best farm inspectors. Laboratory testing can be done by dairy bacteriologists and chemists.

While a four-year college course might fit one to take up this specialized work, graduates in dairying, veterinary science and engineering, as courses are now offered, will find it to their advantage to take graduate work which will better fit them for the complete duties of the milk sanitarian which in the majority of cities in the country they may be called upon to perform. The extra training will also stand out in good stead in the large health departments.

### *Training and Experience for Key Positions*

The selection or advancement of men to key positions should be largely dependent upon experience in the production and handling of milk, the processing of milk and milk products, and in milk sanitation work, together with a demonstrated executive ability.

One Bureau Chief puts it this way: "In my opinion the duties of milk sanitarians should become progressively more of an administrative nature as they gain experience. Beginners should be assigned the simpler tasks such as sample collecting. As they gain experience, they should be assigned to dairy farm and pasteurizing plant inspection, then they may be called on to assist in the investigation of milk supplies under suspicion as responsible for milk-borne outbreaks and to participate in educational programs such as training new inspectors and presenting discussions to the general public. Finally they may be given the full responsibility for the administration of a program in milk sanitation for a municipality, district or state. This would include the making of decisions as to where to place the effort, the assignment of work to subordinates and the establishment of procedures."

### DOES THE COURSE OF STUDY FOLLOWED BY DAIRY MAJORS IN OUR UNIVERSITIES AND COLLEGES ADEQUATELY EDUCATE THE STUDENT TO BECOME A MILK SANITARIAN?

Professor Ely, of the University of Kentucky, in the *Journal of Milk Technology* for May 1938, in summarizing a survey entitled "What the Colleges Are Doing in the Training of Milk Sanitarians," reported an estimated number of 140 four-year graduates in dairying, located in 37 states, were engaged in milk-inspection work. Nevertheless, if we assume that the aforementioned statement on educational requirements represents a fair composite picture of the courses that should be taken by a man planning to become a milk sanitarian, then we would have to conclude that one could not hope to take all of the courses mentioned in four years of undergraduate

work. It seems evident that a special undergraduate curriculum to educate a person to become a milk sanitarian is not warranted by the opportunity that he is likely to have in this profession. The best that can be done is to encourage the dairy major, who thinks he might like to be a milk sanitarian, to elect a course or two in sanitary engineering, some work in public health, some work in veterinary science as related to the anatomy and diseases of dairy cattle and the relation of those diseases to a safe milk supply, a course in dairy engineering which emphasizes the design and construction of farm dairy buildings and processing plants, plan reading, and the sanitary design and construction of dairy equipment.

When one is certain that he is going to make his life work that of a milk sanitarian, he will do well to take graduate work since he will find it impossible to take all of the work that would be of direct benefit to him in a four-year dairy course.

#### OPPORTUNITIES FOR THE DAIRY GRADUATE AS A MILK SANITARIAN

*In the Army.* On December 22, 1943, Major C. J. Babcock, a member of this committee, and your chairman had a conference with General R. A. Kelser, head of the Veterinary Corps. As a result of this conference the following statement has been prepared:

The Medical Department of the Army is directed by The Surgeon General, Major General Norman T. Kirk. There are ten Service Commands in the United States and Alaska. The Medical Department services in these respective Service Commands are under the jurisdiction of a surgeon at Service Command Headquarters. Included in the various Medical Department installations and facilities in each of the ten Service Commands is a laboratory which, in addition to other functions, routinely examines food specimens which are sent to the laboratory by Medical Department officers on duty in the geographical area of the Service Command. These laboratories are well equipped and are conducted by officers of the Medical Department, including Medical Corps, Veterinary Corps and Sanitary Corps. In addition to the Service Command laboratories many of the larger posts, camps and stations have laboratories which are operated in connection with the post, camp or station hospital. Many of these station laboratories are equipped to do bacteriological and chemical control work in connection with milk and dairy products.

The Service Command laboratories are fully capable of meeting all Medical Department requirements not handled by post laboratories. There has been an opportunity for some men trained in dairying, particularly those having had considerable bacteriology and chemistry and laboratory experience, to be placed in both the Service Command and station laboratories, and some dairy graduates are so engaged. Some of these men hold commissions in the Sanitary Corps and some are on duty as enlisted technicians.

The Veterinary Corps, of which General Kelser is in charge, is a branch of the Medical Department operating under the Surgeon General. The functions of the Veterinary Corps fall in two distinct categories—those having to do with the health of military animals, and those pertaining to the inspection of meats, meat-food and dairy products.

The Veterinary Corps is responsible for the evacuation and medical and surgical care of sick and wounded animals; the management and control of veterinary military hospitals and all other veterinary units; investigation of the hygiene and the sanitary condition of the animals of the Army; advising as to methods of animal management; instructing military personnel in animal sanitation and management; and the inspection of forage.

In connection with its food inspection functions, the Veterinary Corps is charged with the inspection of all food products of animal origin, which of course includes milk and its products. Thus, it will be seen that dairy products inspection is not the major part of the job of the Veterinary Corps. Except for a few dairy specialists, there is no such thing as a full time dairy inspector in the Army nor would it appear that there could be, since the bulk of the work that has to be done by the Veterinary Corps is foreign to the experience and training of the dairy student or milk inspector.

Men holding a Reserve Officer's commission as a result of R.O.T.C. training in college when entering the Army are generally assigned to the branch of service for which their R.O.T.C. training qualifies them. While occasionally such a person with dairy training might be transferred for special milk work, this is rarely done.

When the dairy graduate who does not hold a commission is inducted into the Army, he reports along with everyone else inducted into the Army, to an induction center. He then proceeds to a reception center and from there to a replacement training center. Such of these men who go to a Medical Department Replacement Training Center are given a course of basic military training supplemented with special studies to fit them for Medical Department duties. At these medical training centers, the dairy graduate, for example, might, after his training, have an opportunity for assignment to a Service Command or post laboratory, or for work as an assistant to a veterinary officer who would be in charge of food inspection work, including milk and its products. Whether or not any of these men would subsequently have an opportunity of obtaining a commission would depend upon military requirements and vacancies, particular qualification, demonstrated proficiency, etc.

The Reception Centers often have more men with training or experience in a particular field, dairying for example, than there are places in the Army for men with the particular type of training. This, in part at least, explains why the men are not always placed where they feel they can make best use of their experience.

General Kelser explained that in the inspection of milk supplies for the Army it is not the purpose to take over the functions of local health officers or milk control officials. The purpose is to work with local authorities and, where satisfactory conditions are found, the Army's inspection of milk plants is then limited to periodic check inspections to insure that good conditions continue, and to bacteriological, chemical, and other laboratory tests of the delivered milk. In some areas local health authorities for various reasons are unable to do as good a job on their own account as they should, or to cooperate satisfactorily. Where this happens, the Army obviously must give more attention to milk control measures in the area than would otherwise be the case. It is definitely the policy of the Veterinary Corps in their milk inspection work to regularly analyze samples of the product in the post command laboratories and where the results indicate the product is satisfactory, particularly such things as the bacterial count and the coliform count, and the phosphatase test, very little time is spent by a veterinarian or one of his assistants in inspecting the farms where this milk is produced or the plant where it is processed. That undoubtedly explains to some degree why some dairy plant operators feel that veterinarians or their assistants who visit their plants spend little time in them and seem to know so little about product processing. While, as General Kelser explained, it is, of course, true that many of the veterinarians have not had extensive experience in plant processing, they have had basic training in food handling and control. Furthermore, a refresher course is given to groups of twenty to twenty-five veterinary officers each month at the Chicago Quartermaster Depot in Chicago. In these courses some time is devoted to milk and dairy products.

The above should explain why the number of jobs for dairy graduates doing milk and dairy products inspection work in the Army is limited. The inspector apparently has to be an overall purpose animal food inspector; and certainly with the basic training that veterinarians get, it is comparatively easy to train them to do what dairy products inspection is necessary, whereas it would be entirely impractical to train a dairy graduate in what a veterinarian knows about the inspection of meat, eggs, poultry and the care and treatment of animals, etc. The Veterinary Corps will do well in designating men for milk inspection work to try to select men endowed with better than the average amount of practical common sense. Several milk sanitarian associations have voiced their sentiments that qualified milk sanitarians should be used in the armed services whether or not they are veterinarians, but the answer has always been that it is "contrary to policy." Thus a committee of the New York State Association of Milk Sanitarians concludes in their 1943 report, "The Committee now feels that the only approach to the problem is a long-range program of education. The proper recognition of the qualified milk sanitarian should be established in civilian capacity as well as in the armed forces."

*In the U. S. Public Health Service.* The following statement is copied from the Journal of Milk Technology, November-December, 1943:

#### Dairy Graduates in U.S.P.H.S.

"In Mr. Sydney Shepard's article on the educational background of milk sanitarians in the July-August issue, the following statement appears on page 235: 'Today, only sanitary engineers may hold commissions as milk sanitarians with the U.S.P.H.S.' I wish to point out that this was formerly the case but is no longer true. During 1943 a number of dairy graduates, veterinarians, bacteriologists, etc., have been commissioned for active duty on milk and food sanitation in the Public Health Service Reserve, and additional appointments are being processed. Other milk sanitarians on duty under Civil Service may be eligible for commission but prefer not to apply. Of the 30 milk and food sanitarians appointed to date for field duty, 5 are engineers (all commissioned), 4 are veterinarians (2 commissioned), 7 are bacteriologists, biologists, etc. (2 commissioned), and 14 are dairy graduates (10 commissioned). It is evident, therefore, that dairy graduates constitute the largest single professional group of Public Health Service milk sanitarians, both in total number and in commissioned personnel, and that engineers comprise a small minority.

"The question of the proper qualifications for milk sanitarians is indeed a complex problem and one which must be treated with diplomacy and mature judgment so as to avoid apparent favoritism toward one group or another. Persons with several different types of education can and are rendering valuable service in milk sanitation work. The present war conditions demand the best efforts of milk sanitarians as individuals, the proper utilization of the skills of all of them, and above all the greatest possible unity of effort.

"Very truly yours,

"A. W. Fuchs

Sanitary Engineer Director  
in Charge, Milk & Food Unit"

*In City and State Health Departments.* The number of positions carrying considerable responsibility and paying what might be considered an adequate salary that are available in city and state health departments is, of course, limited. Frequently dairy graduates engage in this work and then drift into other fields of work. Professor Ely, in the report above referred to, has found that this was the trend in 20 states. The matter of political pressure on workers and the politics entering into the matter of salary increases were given as the principal reasons why dairy graduates shift from health department work to other fields.

*In Private Industry.* More and more, private industry is carrying on quality control work on milk and milk products, and naturally this is a more attractive field to the dairy graduate from the matter of salary and opportunities for advancement. The fact that up to the time the war started the commercial dairy industry had been able to absorb practically all of the good four-year dairy manufactures graduates to engage in various activities in the business is undoubtedly one of the principal reasons why more of these graduates have not entered or continued to work for public milk quality control agencies.

#### SUMMARY

1. Three definitions of a milk sanitarian are presented.
2. Based on correspondence with several milk sanitarians and the heads of several dairy departments, the duties, general qualifications and the type of education and training for a milk sanitarian have been listed.
3. It is concluded that courses in dairying, as now offered, do not completely educate a student in all of the qualifications of a milk sanitarian. It is felt that a special course to do this is not justified. A student who thinks he might like to engage in milk sanitation work should be encouraged to elect certain courses, along with his dairy work, and if he expects to go far in the milk sanitation field, he will do well to do graduate work so that he can get more of the courses that will be helpful to him.
4. Due to Army policy, there appears to be little or no opportunity for the dairy graduate to be commissioned to do milk sanitation work in the Army. The committee urges that the American Dairy Science Association use its best efforts to improve this situation.\*
5. It is apparent that opportunities for dairy graduates to do milk sanitation work do exist in the United States Public Health Service.
6. There are opportunities for dairy graduates in milk sanitation work in City and State Health Departments but the future which the positions offer and the rate of pay are such as to make the positions rather unattractive.
7. There is more and more opportunity for the dairy graduate to do milk sanitation work in private industry. These positions are attractive because of the wide possibilities of advancement in the industry.

\* See resolution passed by The American Dairy Science Association at the Annual Business Meeting, June 22, 1942.





## THE THIRTY-NINTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, *Secretary-Treasurer*

The American Dairy Science Association assembled in Campbell Hall on The Ohio State University campus at Columbus, Ohio, on Tuesday, June 20, 1944, at 10 A.M.

Dr. Arthur C. Dahlberg, President of the American Dairy Science Association, called the meeting to order, and presented the following officers: Vice-President Arthur C. Ragsdale, Directors P. H. Tracy, C. L. Blackman, R. B. Becker, W. E. Petersen, G. M. Trout, H. P. Davis, Editor T. S. Sutton, and Secretary-Treasurer R. B. Stoltz.

Dr. Howard L. Bevis, President of The Ohio State University, was then introduced and delivered an address of welcome.

President A. C. Dahlberg gave the following response:

### *President's Address*

#### THE APPLICATION OF SCIENCE TO THE DAIRY INDUSTRY

A. C. DAHLBERG

Last year our president, H. P. Davis of Nebraska, gave us a very interesting and scholarly address on the subject of dairy science. It seemed to me to be appropriate to follow his address by one dealing more specifically with the application of science to the development of the dairy industry.

Progress in dairy science and in the dairy industry in this country has been very rapid, yet it might also be considered as very slow, depending upon the point of view. It has been very rapid in that the present modern commercial dairy industry has developed principally within the last fifty years. It is often said that during this period more progress has probably been made than in all previous history. Milk plants, cheese factories, ice cream plants, and condensed and dry milk factories were minor factors in marketing and processing milk and its products over fifty years ago. At that earlier time, within the memory of our older members, the feeding, breeding and management of dairy cattle was primitive indeed when considered in comparison with scientific feeding, herd testing, constructive breeding, and artificial insemination of today. On the other hand, progress has been very slow indeed when one views the situation from the viewpoint of the progress in the establishment of new dairy practices and products of the past half century.

We have been such enthusiastic worshippers of scientific research that there is a tendency to overemphasize the true significance of each individual

discovery and to exaggerate its effect upon the dairy industry. My purpose is to point out that few individual discoveries have materially affected the dairy industry but the total of these little additions to our knowledge has produced continual slow progress. Let us consider the facts, that our prospectus may be more accurate.

### *Dairy Cattle Breeding and Milk Production*

Textbooks written about 1850 to 1890 describe all the present breeds of dairy cattle now important in the United States. Photographs of Ayrshire, Jersey, Guernsey, Holstein, and other cattle show types that can be easily recognized today. Many of the illustrations could be used in modern books but few of these cows would place in present-day cattle shows. Advances in genetics have not developed a new or superior breed of dairy cattle.

The purpose of the dairy cow is the production of large quantities of milk, the only substance produced by nature for exclusive use as a food, and it is surprising how much milk was produced by some of the dairy cows of the preceding century. For example, Dr. E. Lewis Sturtevant, before he became the first Director of the New York Agricultural Experiment Station at Geneva in 1882, was a noted breeder of Ayrshire cattle. A writer of that time wrote about Mr. Sturtevant's herd that "his herd of 65 Ayrshires in June, 1864, produced an average of 33 pounds of milk per cow." This writer also stated that "Good Jersey cows usually give forty pounds of milk per day when fresh and ten pounds of butter per week." These are good records today. Doctor Sturtevant wrote a book, entitled "The Dairy Cow." She was the Ayrshire. In this book are the following statements: "In 1873 General S. D. Hungerford of Adams, Jefferson County, New York, exhibited at the New York Fair at Albany an Ayrshire cow known as 'Old Creamer' whose yield of milk has never to our knowledge been surpassed. Old Creamer is nine years old and weighs 1080 pounds. In three days she yielded the enormous quantity of 302 pounds of milk. . . . She gave 2820 pounds in the month of June; an average of over 94 pounds per day, 2483 pounds in the month of July, an average of over 80 pounds per day." About a decade ago I checked production of the Ayrshire herds of our colleges and I did not locate one cow that equalled or exceeded this production.

Not all cows were good cows then and not all of the best farmers had good dairy cows. There were poor herds then as there are now. To illustrate, George Washington, a master farmer of real ability, wrote to his farm manager a note in which he stated, "It is hoped and will be expected, that more effectual measures will be pursued to make butter for another year; for it is almost beyond the belief, that from 101 cows actually reported on a late innumeration of the cattle, that I am obliged to buy butter for the use of my own family."

The discovery of Mendel's law of inheritance has added to our knowledge but has not materially advanced the breeding and production of dairy

cattle. The present development in proved sires, herd testing, and artificial breeding should greatly increase milk production for the first time in generations. The greatest advances ever made in the improvement of dairy cattle are just commencing. Although some excellent research has been basic to this probability and must continue, the major effort to develop the program now must be in teaching and extension.

### *Dairy Products*

Milk, cheese and butter are products mentioned in the earliest of historic writings. Dry and concentrated milks and ice cream were produced prior to the advance of modern commercial dairying. The question may be asked as to the dairy products that are new in the last half century. These products, such as lactose, casein plastics and cloth, dried whey and others, represent a very minor portion of the dairy industry. No entirely new product of great importance at the present time has been invented.

It would be incorrect even to imply that scientific research has not greatly assisted in the modern, great development of the dairy industry. Present-day dairying is dependent upon the accumulation of scientific knowledge and application to dairy practice.

Perhaps the first modern advancement in scientific dairying that should be mentioned is a practical test to determine the fat content of milk. This test to us is that developed by Dr. S. M. Babcock, but in some other countries it is the test of Dr. N. Gerber. The contribution of Dr. Babcock in perfecting the test consisted chiefly in standardizing the strength of the acid and in developing a pipette to deliver the correct weight of milk for the test bottle employed. He used a centrifuge and a special test bottle, but these were not new. There were several tests then on the market that were more or less successful. Based upon present interpretation of patent laws I doubt that Dr. Babcock's research of his test represented a clear-cut patentable invention. Nevertheless, I believe that this test is absolutely fundamental as the basis for modern commercial dairying, both production and manufacturing, and represents one of the great research developments of modern dairying.

Another development of recent origin that has made modern dairying possible, especially the fluid milk industry, is pasteurization. It is true that Dr. Louis Pasteur applied his process to wine fermentations in 1864 but he did not apply it to milk, and its application to milk and milk products by others is of more recent origin. In this connection it is interesting that some books whose publication precedes the pasteurization of milk actually mention that scalding milk improved its keeping quality. Without pasteurization the maintenance of safe dairy products of good quality would not be possible as they need to be handled on a large-scale basis.

The butter industry became a major branch of commercial dairying after the invention of the cream separator made it possible to eliminate gravity

skimming. The chief contribution of Carl Gustaf DeLaval that made the centrifugal separator possible was the devising of a procedure to make the process a continuous one. DeLaval was not the first to remove the cream from milk by centrifugal means. The continuous cream separator made it possible to have a large factory system of manufacturing butter and is, therefore, one of the great developments of the dairy industry. It is of recent origin.

Thus, the three great developments of the dairy industry of modern times aided recognized products and processes rather than created new ones. There are several products recently developed by research that are very important factors in the dairy industry. Process cheese by its uniformity, keeping quality, and packaging has created new markets for cheese. Ice cream, concentrated milk, and dry milk are largely the result of a mass of scientific research and practical developments which has made them as they are today. Although Gail Borden is said to have invented condensed milk, he actually made it commercially feasible through the exclusion of air during concentration and the use of milk of good quality. Most of our major dairy products have been developed through much research and development and not through revolutionary scientific research.

Progress on basic foods is seldom rapid. One of my purposes in this discussion is to emphasize that progress must be slow in the advancement of the production and processing of an agricultural good like milk and its products. The research program must be extensive, yielding information on many aspects of the subject. Some of the new facts explain why processes must be as they are, others are informational only, while more limited facts are utilized to advance some phase of our industry. Each contribution in itself may seem small, yet each to a greater or a lesser degree adds to dairy knowledge to improve both science and industry. This should encourage us all to do our bit to the best of our ability with full knowledge that it rarely happens that a discovery is made that greatly affects the industry but that the mass of knowledge means progress. Actually more effort is often required to adapt and to promote the newer knowledge into the dairy industry than was required to discover it.

In the fields of chemical and physical research the results often produce revolutionary products and processes that affect our whole nation. Automobiles, radios and plastics are examples. Such research stimulates our imagination and brings new industries and products into our daily lives, but such research never has revolutionized the dairy industry. Such new industrial products then require manufacture and merchandising, but in the dairy industry there is need for an immense effort in teaching and extension to promote the newer knowledge as it applies to our older products. Such is the case for the discoveries of the excellent nutritive properties of dairy products for they do not create new products but require education of the people to use more dairy products for their improved nutrition.

Then too, the margin of profit in the dairy industry is too small to permit most extensive research and development of entirely new products. The business from the farm to the consumer is so fixed in routine, regulations and margins of profit that it is more difficult to introduce radically different products. Some dairy companies are sufficiently large to maintain excellent research laboratories. Such research must be confined largely to projects which show promise of immediate practical application for it is not apt to be true in dairy research that studies of pure science may yield greatest dividends as so often occurs in chemical and physical research laboratories. Advances in pure science sometimes advance dairy science which in turn may improve our dairy practices.

Dairy research for both dairy farmers and manufacturers of dairy products must be conducted chiefly by public research agencies supported by private and public funds. Private funds will need to be given more and more from current earnings rather than through inheritance as has been done in the past. Some excellent long-time dairy-cattle projects could be endowed in this manner. It would be most unfortunate for the public if the advances in dairy products were limited largely to the research of the larger dairy companies, important as their research may be. There is need for public research in food processing of the most important foods in the United States when viewed from both nutritional and financial standards, namely, milk and its products. It is only through such research that the entire industry may advance to the fullest extent. Our legislatures and agricultural leaders often overlook this fact. We have tended to take this advancement for granted because it has occurred in the past, or we have said that the dairy industry could serve itself by applying fundamental science to its problems. This application is often much more difficult to solve than the problem in fundamental science.

### *The American Dairy Science Association*

As President of our Association it is proper in conclusion to comment briefly upon some aspects of its work and development. The Association has served well in many respects in aiding dairy science and the dairy industry. We have been rather modest in the publicity given to the work of the Association. This is undoubtedly a desirable virtue, yet it has tended to minimize somewhat the Association, particularly in the views of scientists in other professions.

The tremendous amount of effort of the many committees of our Association is scarcely appreciated even by our own membership. The accomplishments of these committees are the reward of its members for their efforts. For example, consider the valuable work of the committee on the testing of dairy cattle for production and of the committee on standardization of technic of the Babcock test. Their recommendations are standard

procedures. Our Association serves as a common meeting ground to present the work of our members. In normal times and in recent years, the annual meeting has been a combination of scientific work and social fellowship that has been most conducive to good work and cordial relationships.

The work of our Association must be centered in the Editor of the Journal and the Secretary-Treasurer. Both tasks require much effort and must be done extremely well by men of training, temperament and ability to do the work. Furthermore, both tasks must be done chiefly for the good of the Association, dairy science, and the dairy industry, for neither office is paid a salary sufficient to attract men of the ability required to do the work. The work of the Editor as shown in the scientific standards of the Journal of Dairy Science is a direct reflection on the character of our membership, which means you and me. It is brought to our attention and to all readers each month as Journals arrive. The work of the Secretary is not so obvious. He must guide our Association under the supervision of the Executive Board. It is no accident that our membership has increased about fourfold in the last fifteen years; that our income from advertising has increased about fivefold in this same time; that the financial reserve of our Association has increased from nothing to an amount equalling a full year's income; and that our membership fee has not changed while the size of our Journal has more than quadrupled. These results are due in part to continual effort on the part of the Secretary for which he is paid for all his services the equivalent of a reasonable rate that would be charged by an advertising firm to secure the advertising alone. Our Association is fortunate indeed to have the services of our present Editor and Secretary. May we give them partial compensation for the splendid services which they render to our Association by our appreciation and loyalty.

Let no one assume that our Association has reached its goal, its maximum development, and from now on we shall coast easily along. We must progress or we shall go backward. I well remember when we were struggling to secure a membership of 400; now we have a larger attendance than that at our annual meetings. After this war there is an opportunity for a greatly increased sale of the Journal of Dairy Science outside the United States. Now our foreign subscriptions are subnormal. Our Journal is a leader in its field, it is improving, and these foreign workers need it more than ever before. After the war there should be a material increase in the membership in our colleges. Men in commercial work have been joining our Association in increased numbers and they are especially welcome. These men are in research, production, and extension. Membership in the American Dairy Science Association of college workers in the research, teaching, or extension in all phases of the dairy industry is almost obligatory to keep abreast of the times. Our college men must be leaders in dairy education. The future of our Association is very promising.

Mr. Games Slayter, Vice-President in charge of research and development for the Owens Corning Fiberglas Corporation, of Newark, Ohio, was then introduced and gave a very stimulating address, bringing out the need for more research in this country if we may expect to compete with other countries.

President Dahlberg then announced the Resolution Committee:

FORDYCE ELY, *Chairman*

H. F. JUDKINS

I. R. JONES

C. R. GEARHART

H. P. DAVIS

He also announced that the Directors had accepted an invitation to meet at Iowa State College at Ames, Iowa, in 1945.

There were 233 members present. The meeting adjourned at 11:40 A.M.

## GENERAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

*Columbus, Ohio, June 22, 1944*

President Dahlberg called the meeting to order at 3:30 P.M. in Campbell Hall, there being 95 present.

### EDITOR'S REPORT

The Editor begs to submit the following brief report to the membership of the Association:

A summary of the Journal contents for the past five years is in the accompanying table. These figures are on a comparative basis and include the contents of twelve issues, July to June inclusive.

It will be noted that, in spite of war conditions, contributions to our Journal have held up remarkably well. The number of original manuscripts published during the past year is exceeded by that of only one previous year, 1941-42. The articles were of somewhat shorter average length, and fewer pages were devoted to review articles resulting in a decrease of 210 printed pages during the past year.

Only one review article was published during the year; another has been received and will appear in the July issue. Several others are reported to be in some stage of preparation.

The number of pages of abstracts has fallen off slightly. This is not a reflection on the effectiveness of our abstracting organization but rather a result of a little finer sifting and a decrease in the quantity of published material of interest to the dairy industry.

Again your Editor wishes to acknowledge the cordial spirit of helpful cooperation of all those who have assisted in the discharge of editorial responsibilities.

## SUMMARY OF JOURNAL CONTENTS

	1939-40	1940-41	1941-42	1942-43	1943-44
Number of original articles .....	94	97	101	89	99
Pages of original articles .....	826	892	938	788	706
Number of reviews .....	3	5	3	2	1
Pages of reviews .....	120	144	80	142	64
Miscellaneous .....	212	278	106	192	206
Announcements, Proceedings, Circulation, Index, etc.					
Pages of Abstracts .....	206	304	418	290	226
Total number of pages printed .....	1454	1618	1542	1412	1202

*Classification of Articles*

Manufacturing articles .....	53	56	59	50	52
Pages occupied by Manufacturing .....	450	514	548	414	480
Production articles .....	31	35	36	32	42
Pages occupied by Production .....	296	216	336	314	325
Manufacturing-Production .....	10	6	6	7	5
Pages occupied .....	80	62	54	60	46
Manufacturing reviews .....	0	5	1	1	1
Pages occupied by Manufacturing reviews .....	0	144	36	86	64
Production reviews .....	2	0	2	1	0
Pages occupied by Production reviews .....	120	0	44	56	0

Upon motion duly seconded the report was accepted.

## SECRETARY-TREASURER'S REPORT

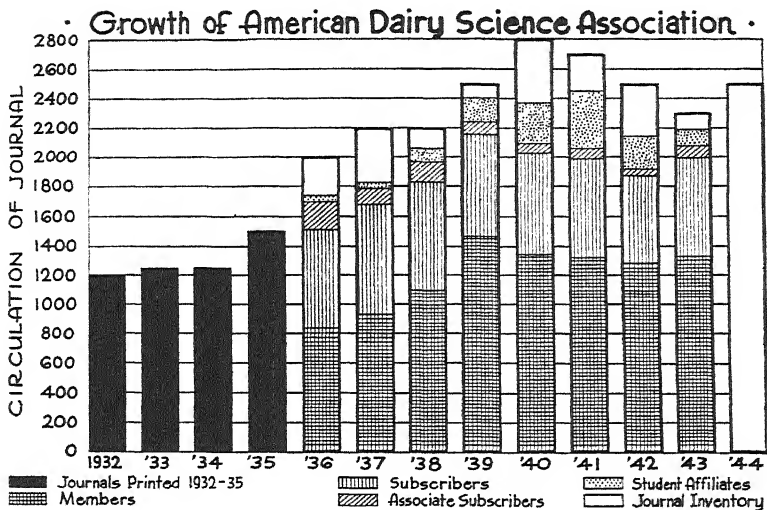
*Membership and Circulation.* The circulation of the JOURNAL OF DAIRY SCIENCE for the year 1943 was very satisfactory. We had 1324 members, which is the highest membership we have had, with the exception of the years 1939 and 1940. Without putting on an organized campaign, we received 134 new members, 83 of which paid the \$5.00 affiliation fee and 51 of which were former student affiliates.

The domestic subscribers amounted to 463, which is almost 100 more domestic subscribers than the Journal has ever previously had. Our foreign subscribers were 208, which is the lowest we have had in the last seven years. We had 85 associate subscribers, which is the highest since 1938, and 108 student affiliates, which is the lowest we have had since 1938, making a total circulation for 1943 of 2,197. The chart shows the growth of our circulation over the past twelve years. The height of the current column indicates the number of Journals that were printed. We anticipated a decreased circulation in 1943 over 1942, but our membership and domestic subscribers both increased to such an extent that the dropping off of the foreign subscribers and student affiliates was more than overcome. Thus far in 1944 our circulation is ahead of last year's at the same time by more than 100. As of June 17 our membership this year is 1281 while last year at the same date it was 1245. Our domestic subscribers are 467 while last year they were



402. Our foreign subscribers this year are 206, and last year they were 166. Associate subscribers are 78 versus last year's number of 64. Student affiliates this year are 39 and last year 100. The total circulation on June 17, 1944, is 2071 and last year at the same date it was 1977.

Our delinquents this year are less than usual, but we may always expect to lose approximately 10 per cent of our membership annually. Therefore, in order to hold our present circulation, it will always be necessary to be on the alert for new members and subscribers. After the duration our circulation list should be at least 3,000 and in the future our new members should largely come from our student affiliates. Your Directors have taken action



which will permit student affiliates who were in the military service to become members without paying an affiliation fee at the end of the duration.

During 1943 we had 2300 Journals printed, thinking that the circulation would decrease at least 200 under 1942. Instead of this our circulation was higher in 1943 than in 1942; consequently, all the Journals that were printed were sold and it was, therefore, necessary for us to advertise and buy back the 1943 Journals. We were able to purchase 58 volumes for \$173.50, 11 of which have been sold, so that our present inventory will take care of the needs for the next few years.

*Back Copies.* We are now able to supply all the back copies of the Journal because the Board of Directors have reproduced 13 numbers at the cost of \$1710.18. We have approximately 40 or more volumes of each year, with the exception of Volume XXII (1939). During 1943 we sold \$1133.85 worth of back copies, while in 1944 up to June 1 we have sold \$1122.25, which included eight sets of all back Journals.

The following pamphlet "To Readers and Authors of Scientific Papers" was sent to our membership last fall: "The Officers of your Association are anxious to have you read the following editorial written by the Editor. He is attempting to get the authors to write the material in your language and asking you to increase your vocabulary, so that you will understand it. We commend this article for your reading. Signed, A. C. DAHLBERG, President, American Dairy Science Association.

*Your Journal. Keeping Abreast with Progress*

"The JOURNAL OF DAIRY SCIENCE made its first appearance in May, 1916. At that time the American Dairy Science Association felt the need of a scientific journal devoted to dairying to serve as a place for the publication of the researches of its members. The science of dairying was then in its infancy and those engaged in research and teaching were directly interested in all phases of the subject. Great developments were in the offing, however, and the rapid advances which were soon to follow surpassed even the dreams of those who pioneered in the field. The JOURNAL OF DAIRY SCIENCE takes modest pride in the part it has played in the progress of dairying.

"Dairying is an applied science rooted in the fundamentals of chemistry, physics, biology, etc. As the scope of interest broadened, specialization became a necessity. Today the broad field of dairying includes a wide diversification of interests. This diversification of specialized interests has brought about changes in the JOURNAL OF DAIRY SCIENCE. Today, it would be unusual indeed if every original article in a single issue was of direct interest to every member of the Association. Every volume, however, contains a wealth of material for everyone engaged in the industry. Since the material printed presents new facts and new applications of established facts not previously published, a single article may have inestimable value.

"With the more direct application of the principles of fundamental science to the problems of the industry, the vocabulary and phraseology of the articles appearing in your Journal has changed.

"For example, the researcher, who is making use of his knowledge and skills of chemistry in solving a problem of the industry, will quite naturally use the vocabulary and phraseology of the chemist in reporting his work. Accuracy and precision are essential and at times may be obtained only by the use of scientific terms.

"On the other hand, the use of scientific language may kill the interest of the practical man who could make immediate use of the findings reported. Many scientific researchers claim to have no interest whatever in the practical application of their findings. Yet, were it not for these practical applications, the social system, which supports these fundamental researches, would soon see no need of their continuation and withdraw their support. Simplicity has great virtue, and the presentation of results in understandable terms need not detract from the scientific value of the work.

"The practical man is not without responsibility in this connection. It is his obligation in supporting progress to rapidly become familiar with the language of his profession. Each new development brings new additions to our vocabulary. At first, these new additions may be confusing but many of them are destined to become everyday household terms.

"The policy of the JOURNAL OF DAIRY SCIENCE must be '*constantly forward*.' Real progress can only be measured by the accomplishments of the group as a whole, not by the few leaders who form the 'advance patrol.' Your Journal is dedicated to the purpose of keeping you fully informed concerning the activities of those who through their researches are advancing the science of dairying. You are then in a favorable position to carry out the everyday affairs of the industry with greater dispatch and efficiency. Signed, T. S. SUTTON, Editor."

On the back of the pamphlet is the following notice: "A Library for Each Laboratory. Wouldn't it be a smart move to have all the back volumes of the Journal with the 20-Year Index in every commercial dairy laboratory? Don't you think every milk, ice cream, evaporated milk and powdered milk plant should have a library as much as it should have a receiving vat or compressor?"

"You may not have anyone who has time to read now, but this may be the proper time to procure the backbone of your library, which should be the 26 volumes of the JOURNAL OF DAIRY SCIENCE, with the 20-Year Index. The cost is \$145.50. If you're paying 90 per cent taxes on excess profits, it makes your library quite a low cost."

During the year a pamphlet entitled "*Opportunities in the Dairy Industry in the Post War Period*" was offered to schools to be furnished to the graduates and undergraduates in the service: "The Officers of the American Dairy Science Association have prepared this pamphlet for the benefit of the boys in the Service who have had some Dairy Science Training. Additional copies may be obtained by writing the office of the Secretary. Signed, A. C. DAHLBERG, President, American Dairy Science Association.

"In keeping with the purposes of the American Dairy Science Association we are interested in encouraging all the boys in the service to continue their college education at their earliest possible opportunity.

"There is going to be a greater demand for trained men than ever before. There has never been an over-supply of trained dairymen, and now there will be an even greater shortage. There are so many things that will need to be done. The dairy industry is still young, and the surface has barely been scratched.

"1. Dairying has developed from a farm dairying practice to a profession.

"2. Each year brings a greater general recognition of the necessity of dairy products in the diet and greater demand for them.

"3. Expansion of the dairy industry is inevitable because of the greater appreciation of the nutritional value of milk and its products.

"4. New processes will be developed which will require the best technical training.

"5. More emphasis will be placed upon efficiency in both manufacturing and production of dairy products.

"6. There is every indication of a great expansion in research both private and tax-supported.

"7. Practices in the production field have taken a technical turn requiring highly trained personnel. Example, artificial insemination.

"8. Many from the technical staffs of our public educational and research institutions have gone into commercial work. Many of these will not return to their old jobs following the war. Replacements must be found among those who finish their training after the war.

"9. In every post-war period the nation has previously encountered, the necessary readjustments 'spot-lighted' dairying on farms and commercially. International adjustments will be important after this war.

"10. Present war experiences have opened a new outlook on the need for advancement in our production and processing methods; especially in preservation, storage, packaging, and new products.

"The American Dairy Science Association believes that the dairy industry will demand far more professionally trained men in the next decade than will be available. Therefore, we urge those who have some training, who like the profession, and who are enthused about its future, to return to college to complete their course or for a refresher course."

*Advertising.* Our advertising income is still on the increase. In 1942 we sold 143 $\frac{3}{4}$  pages, which was higher than any previous year. In 1943 we sold 171 pages. Our advertising for the first half of 1944 is equal to 1943. We are very anxious to again express our gratitude to the companies that use our pages for advertising. Any courtesies shown these advertisers will be much appreciated.

*Financial.* The income for 1943, amounting to \$19,617.26, is the largest of any year during our organization. Our expenditures, amounting to \$18,539.61, leave an operating profit of \$1,077.65, which more than wipes out the deficit of \$1,052.20 made in 1942.

As of December 31, 1943, our net worth was \$22,645.56 and we had an investment in Government bonds of \$19,690. A complete report of the Certified Public Accountant was sent to each Director and Officer in February of this year.

The Secretary-Treasurer wishes to take this opportunity of expressing his gratitude and thanks to the officers and members for the splendid cooperation that has been received.

Respectfully submitted,

R. B. STOLTZ, *Secretary-Treasurer*

## AUDITING COMMITTEE

The President then requested a report of the Auditing Committee, which was given by Mr. L. H. BURGWALD, *Chairman*.

March 27, 1944

To the Members of the American  
Dairy Science Association  
Gentlemen:

Mr. Walter Burnham, of Columbus, Ohio, Certified Public Accountant, has made an audit and report of the financial condition of the Association.

The Auditing Committee has conferred with Mr. Burnham and is satisfied that he has made a careful examination of all assets and liabilities of the Association and that all the accounts are accurate. The committee is satisfied that the balance sheet and related summary of profit and loss represents the financial condition of the American Dairy Science Association.

Respectfully submitted,

L. H. BURGWALD, IVAN MCKELLIP, H. E. OTTING

Mr. Burgwald moved and Mr. Blackman seconded that the Auditing Committee report be accepted.

Mr. D. M. Seath moved and Mr. W. L. Clevenger seconded that the Secretary-Treasurer's report be approved.

## JOURNAL MANAGEMENT COMMITTEE REPORT

Columbus, Ohio  
June 19, 1944

Executive Board  
American Dairy Science Association  
Gentlemen:

This has been a good year for the JOURNAL OF DAIRY SCIENCE from the viewpoint of the Committee on Journal Management.

The President of the Association, the Secretary-Treasurer, and the Editor of the Journal have functioned so efficiently in their respective capacities that but few problems have confronted the Committee on Journal Management during the year.

Among certain items that have received the attention of the Committee was the question of publishing news items in the JOURNAL OF DAIRY SCIENCE. The Executive Board had referred this matter to the Journal Management Committee for consideration, inasmuch as there had been some agitation for such news items in recent years. President Dahlberg, in submitting the problem for the careful thought of the Committee, called to attention the fact that the JOURNAL OF DAIRY SCIENCE once did publish news items and that they were discontinued largely because they were so incomplete and unsatisfactory. When he first became editor of the Journal he appointed a news reporter in every Dairy Department in every State but satisfactory

results did not materialize. He suggested that if the news item feature were adopted a special effort will be needed to make such a service satisfactory to our members.

After careful consideration, the Journal Management Committee was inclined to disfavor this venture for the following reasons:

1. The news item feature, attempted once before, had been discontinued because of lack of interest among those responsible for submitting the news. The editor has had a similar experience in connection with abstracting work. At one time there was a person at each experiment station charged with the responsibility of abstracting station publications of his own experiment station. This arrangement proved so unsatisfactory that finally it became necessary to adopt other means of securing abstracts.

2. If news items that are considered suitable by certain members of the organization were not regarded as of sufficient academic and general interest by the editor to warrant publication, embarrassment or friction might result.

3. A majority of Journals somewhat similar in their field to that of the JOURNAL OF DAIRY SCIENCE in the dairy industry do not publish news notes. The strictly scientific journals seem to carry no news notes at all.

In view of the foregoing, the committee felt that it would not be wise to attempt a news feature at this time. However, should a strong demand for a news section arise among the membership at a later date, the matter can then be given further consideration.

*Ten-Year Index.* The Committee on Journal Management has given consideration to the advisability of publishing a 10-year index for the period ending in 1946, and would recommend to the Executive Board that immediate steps be taken toward the publication of a 10-year index of the JOURNAL OF DAIRY SCIENCE for the years 1937-46.

*Replacements on Editorial Board.* The Committee also recommends that the Editor give consideration to a revision of the list of Associate Editors in order to have a more representative group.

R. B. STOLTZ, T. S. SUTTON, FORDYCE ELY,  
G. M. TROUT, A. A. BORLAND, *Chairman*

#### PRODUCTION SECTION

Secretary D. M. Seath, of the Production Section, presented the following report. He moved and Mr. M. O. Maughan seconded that the report be accepted. Motion carried.

This year's meetings of the Production Section were well attended. One joint symposium was held with the Extension Section on the general subject of "Mastitis and the Feed Situation" with special emphasis on their relationship to the war effort. On Tuesday and Wednesday papers relating to research problems in the various states were presented in two simultaneous sessions of the Section. Papers thus presented totaled 44.

Two business meetings were held. The following committee reports were presented.

The Report of the Dairy Cattle Breeding Committee was read and approved. During the past six years this Committee has spent considerable time in the preparation and revision of recommended procedures for the organization and operation of artificial breeding associations. It has also worked with the Purebred Dairy Cattle Association in evolving regulations for the safeguarding of registrations. These procedures and regulations are now contained in a mimeographed circular, which can be obtained from the Bureau of Dairy Industry.

The Dairy Cattle Breeding Committee also approved the inclusion of type rating in conjunction with the reporting of lactation records in the proving of bulls and urges that more attention be given to this phase of the herd improvement program. In view of the present feed situation the committee also urged that the stringent culling of low-producing cows be again emphasized.

The report of the Breeds Relation Committee was read and accepted. In this report were several suggested changes regarding the conduct of Herd Improvement Registry and Advanced Registry tests. Some of these suggestions aim to eliminate conditions arising from the present emergency.

During the coming year a representative of this Committee will serve on a committee of the Purebred Dairy Cattle Association to "write one set of uniform rules" for the testing of all breeds and to study the possibility of improving the testing program.

The Breeds Relation Committee will continue to arrange for the printing and distribution of uniform official testing forms.

Report of the Feed and Pasture Investigations Committee recommended that a concerted effort be made to secure more and better pastures and high-grade roughage. Special emphasis was given to the need for pasture fertilization, and it was pointed out that larger quantities of nitrogen and phosphorus fertilizers are to be available this year. It was stated that there is a need for more information concerning effect of fertilization on palatability and the feeding value of pastures and forage crops. Recommendations were made that extension agencies stress pasture and forage crop production in an effort to produce a larger proportion of feed nutrients from roughage.

H. W. Cave, of Oklahoma, read a report submitted by the Committee on Animal Health of the National Research Council. This report was entitled "Adult Vaccination Against Brucellosis in Cattle." The report was accepted. The Production and Extension Sections asked that the Secretary of the American Dairy Science Association be requested to convey to the Committee on Animal Health of the National Research Council the appreciation of our Association for this progress report and urge the Committee on Animal Health to continue their study of this problem.

The Section voted to replace the feeds committee by creating a new one, called the Feeds Relations Committee, which will handle questions relative to concentrated feeds and especially those questions relating to the feed industry. Such a committee is to be a joint one with the Extension Section as soon as feasible. It was also voted to create a Herd Health Committee that will work jointly with a similar committee of the Extension Section.

The Resolution Committee reported no new resolutions, and that those of a general nature were being handled by Association's Resolution Committee for the general session.

The report of the Nominating Committee was approved and Dwight Seath, of Louisiana, was elected vice-chairman and George Wise, of South Carolina, as Secretary. Glenn Salisbury, of New York, the present vice-chairman, automatically becomes Chairman of the Production Section for this next year.

Respectfully submitted,

DWIGHT ESPE, *Chairman*

DWIGHT SEATH, *Secretary*

#### EXTENSION SECTION

Secretary W. T. Crandall reported the minutes of the Extension Section. He moved and Mr. Morley seconded that the report be accepted.

The Extension Section held all of its scheduled sessions with an attendance of over fifty members from 19 states. At the first session, Chairman Scheidenhelm announced the resignation of Gerald Heebink, of West Virginia, as Secretary of the Section and W. T. Crandall, of New York, was elected to fill the vacancy.

At the first sectional session problems involved in carrying on D.H.I.A. work were discussed by a four-member panel, the 4-H Club program was given, and a report of the 4-H Club adopted.

At the second session papers were given on the "Forced Ventilation System of Curing Hay in the Mow," "Getting Quality Bulls and Quality Semen," and "An Action Program for Rapid Washing Methods." Eighteen State exhibits on new Extension programs or successful methods of teaching were explained and discussed.

A report from the Resolutions Committee was read and adopted.

A joint symposium was held with the Production Session and presided over by Dwight Espe, of Iowa. The problems of Mastitis Control were discussed and a very comprehensive statement of the feed situation was made by Walter Berger, Chairman of the Feed and Livestock Division of the Food Production Administration.

At a joint business meeting of the Extension and Production Sessions reports of the following joint committees were read and adopted:

1. The Dairy Cattle Breeding Committee.



2. The Breed Relations Committee.
3. Pasture and Roughage Committee.

A report on "Adult Vaccination Against Brucellosis in Cattle" by the Committee on Animal Health of the National Research Council was read by H. W. Cave, of Oklahoma. This report was made to the American Dairy Science Association at the request of the 1943 Resolution Committee of the Association.

At its business session, the Extension Section listened to reports by the following committees:

1. Testing Committee.
2. Quality and Marketing Committee.
3. States with Exhibits and Committee on Teaching Ideas.
4. Type Rating Committee.
5. Dairy Cattle Health Committee.

All of these reports were adopted.

The final order of business was the election of E. H. Loveland, of Vermont, as the incoming Secretary for 1944-45. The chairman for the coming year is Floyd J. Arnold, of the University of Maryland.

Respectfully submitted,

W. T. CRANDALL, *Secretary*

#### MANUFACTURING SECTION

Secretary I. A. Gould then gave the following report for the Manufacturing Section. He moved and Mr. Seath seconded that it be adopted.

The programs for the Manufacturing Section were held during the afternoon of Tuesday, June 20, the morning of Wednesday, June 21, and the morning of Thursday, June 22, with P. F. Sharp as general chairman. Thirty-eight titles were accepted for the program, and only four of the papers (M7, M9, M26, M37) were not given. In addition to the regular papers, five additional papers were presented at the symposium dealing with Dehydrated Milk and Milk Products held the morning of Thursday, June 22.

The large number of papers submitted for the manufacturing program necessitated the scheduling of two sections, which met simultaneously. The various sectional programs were entitled Bacteriology, Chemistry, Butter and Cheese, and Milk and Milk Products.

The business meetings of the Manufacturing Section were held Wednesday and Thursday mornings. The Standing Committee reports were presented and accepted from the following committees:

- (a) Committee for Milk and Cream—H. L. TEMPLETON, *Chairman*.
- (b) Committee on the Judging of Dairy Products—G. M. TROUT, *Chairman*.
- (c) Committee on Ice Cream—C. D. DAHLE, *Chairman*.
- (d) Committee on Cheese—E. C. REICHERT, *Chairman*.

Committees on By-Products, Dried Milk, Evaporated Milk, Butter and Ice Cream presented no reports.

Discussions were held relative to man-power shortages, but no final definite action was taken.

The Resolutions Committee submitted no resolutions.

The Vice-Chairman for the past year, L. K. Crowe, of Nebraska, automatically becomes Chairman for the coming year. The two new officers elected were C. L. Hankinson, of Milwaukee, Wisconsin, Vice-Chairman, and N. S. Golding, Washington, Secretary.

Respectfully submitted,

I. A. GOULD, *Secretary*

#### NATIONAL RESEARCH COUNCIL REPORT

Mr. O. E. Reed, who represents this Association on the activities of the Division of Biology and Agriculture of the National Research Council, then told what the Council had been doing during the past year. He presented a list of the present committees, including those of the Food and Nutrition Board. A few of these special interests, which he singled out for individual mention, were

- (1) The Committee on Milk of the Food and Nutrition Board.
- (2) The Committee on Fats.
- (3) The Committee on Dietary Allowances.
- (4) The Committee on Animal Nutrition.
- (5) The Committee on Animal Health.
- (6) The Committee on Food Composition.
- (7) The Food and Nutrition Board.

The report listed the organization of the Division and the basic organization of the National Research Council.

Mr. Glenn Salisbury moved and Mr. Sutton seconded that the report be accepted.

#### TRAINING AND EMPLOYMENT OF MILK SANITARIANS

P. H. Tracy then presented a report on "Training and Employment of Milk Sanitarians," which appears on pages 691-700 of this issue.

Tracy moved and A. A. Borland seconded that the report be adopted.

#### NECROLOGY COMMITTEE

Mr. N. N. Allen of Vermont read the report of the Committee on Necrology as follows:

The Necrology Committee reports the following members as having passed away during the year:

Professor A. C. Anderson passed away on January 13, 1944. Born in New York he graduated from Michigan State College in 1906. He was head

of the Dairy Department of that institution from 1910-1923 when he joined the staff of the Michigan Milk Producers' Association from which he retired in 1940. Professor Anderson was vice-president of the American Dairy Science Association in 1916 and president in 1917, to which Association and the dairy industry he contributed much.

Doctor M. J. Dorcas of Berea, Ohio, widely known for his contributions in the field of science, passed away August 11, 1943. Born in Iowa, he was graduated from Baker University and received his Ph.D. degree from Harvard, following which he taught chemistry at Wesleyan University, in Connecticut, and conducted research at the Bermuda Station of the Rockefeller Institute for Biological Research. In 1925 he entered the Research Laboratories of the National Carbon Company, Cleveland. In later years his efforts were devoted largely to special applications of carbon and graphite products in the chemical and metallurgical industries and to the supervision of construction of synthetic rubber plants.

Edwin A. Hanson, Assistant Professor and Extension Dairyman at the University of Minnesota, passed away on April 13, 1944. Widely known among dairymen of Minnesota and adjoining states, he pioneered in the organization of cow testing and dairy herd improvement associations, took an important part in teaching dairy improvement to 4-H members and was one of the authors of "Feeding the Dairy Herd," a widely used handbook of dairy management in his state. He became Extension Dairyman in 1922.

Doctor Leroy S. Palmer, eminent dairy scientist and Chief in the Division of Agricultural Biochemistry of the Department of Agriculture of the University of Minnesota, passed away on March 8, 1944. Born in Illinois he received his B.S., M.S., and Ph.D. degrees from the University of Missouri where he served on the teaching and research staff from 1911 to 1919, when he went to the University of Minnesota. During more than 30 years of research and teaching he earned world-wide recognition as a chemist, especially in dairy science and nutrition, his principal interests being in such fields as the pigments of milk and butter, cause of butter defects and storage troubles, the physical and colloidal chemistry of milk and the churning process. Dr. Palmer was the first recipient of the Borden Award for outstanding research in the chemistry of milk.

Doctor Elmer S. Savage, professor of Animal Husbandry at the New York State College of Agriculture, passed away on November 22, 1943. He was born in New Hampshire and received his B.S. degree from the University of New Hampshire and his M.S. and Ph.D. degrees from Cornell University with which he has been associated since 1909. Maintaining outstanding leadership in the field of feeding and management of dairy cattle, he was an ardent exponent of "open formula" feeds and contributed much to the development of more economic methods of raising dairy calves through the use of whole milk substitutes. Professor Savage has served as secretary and vice-president of the American Society of Animal Production.

The Committee has just learned that Keith Thorneloe of the Comico Products Corporation passed away in October, 1943, and Mr. O. G. Simpson of Portland, Oregon, passed away in February, 1944.

Mr. N. N. Allen moved and Mr. K. G. Weckel seconded that the report be accepted.

#### RESOLUTIONS COMMITTEE REPORT

Mr. Fordyce Ely, Chairman of the Resolutions Committee, submitted the following report:

**WHEREAS:** The Borden Company has again seen fit to offer their awards for outstanding research in dairy production and dairy manufacturing,

Therefore, be it *Resolved*: That the American Dairy Science Association express to them our sincere appreciation of this evidence of their great interest in research, which is so vital a part of our industry.

**WHEREAS:** The Committee on Animal Health of the National Research Council has presented a report dealing with the problem of Brucellosis vaccination in cattle,

Therefore, be it *Resolved*: That the American Dairy Science Association express its appreciation for the information furnished in said report.

**WHEREAS:** The Committee on Animal Health of the National Research Council has presented a report entitled "Adult Vaccination against Brucellosis in Cattle—A Report to the American Dairy Science Association,"

Therefore, be it *Resolved*: That the American Dairy Science Association request the same committee to prepare a parallel report dealing with the public health aspects of Brucellosis.

**WHEREAS:** There exists a lack of uniformity between States in the interpretation and application of the calfhood vaccination plan for eliminating Brucellosis from herds and since this condition has caused a great deal of confusion and uncertainty,

Therefore, be it *Resolved*: That the representative of the American Dairy Science Association to the Inter-Association Council for Animal Disease and Production be instructed to call this matter to the attention of said Council and to encourage the adoption of uniform procedures.

**WHEREAS:** The inspection of milk and its products supplied to the Army is now vested in the veterinary corps of the Army Medical Department, thereby making it virtually impossible for college or university graduates in dairying, who are well qualified for milk inspection work to obtain commissions in this field,

Therefore, be it *Resolved*: That the American Dairy Science Association petition the proper Army authorities for a change in policy that will make it possible for the qualified dairy graduate to be commissioned to inspect milk and its products, thus making it possible for him to use his talents to the best of advantage in the armed forces.

WHEREAS: Ohio State University, through its administrative staff, its faculty, and especially its Dairy Technology and Dairy Husbandry staffs, have made available to the American Dairy Science Association in this its 39th Annual Meeting all needed physical equipment for the handling of the meetings, and

WHEREAS: In addition every possible personal courtesy has been extended to visiting members of the Association by members of the staff of the University, the staff of the Experiment Station, and local members of the dairy industry, and

WHEREAS: The efficient functioning of the groups mentioned is in a great measure responsible for a most successful and enjoyable meeting,

Therefore, be it *Resolved*: That the American Dairy Science Association take the occasion to officially express its thanks and appreciation and the President of the Association instructed to express this appreciation by letter to President H. L. Bevis and Dean J. F. Cunningham, of the College of Agriculture.

H. P. DAVIS, I. R. JONES, H. F. JUDKINS,  
C. R. GEARHART, FORDYCE ELY, *Chairman*

Mr. Ely moved and Mr. Ragsdale seconded that the report be accepted.

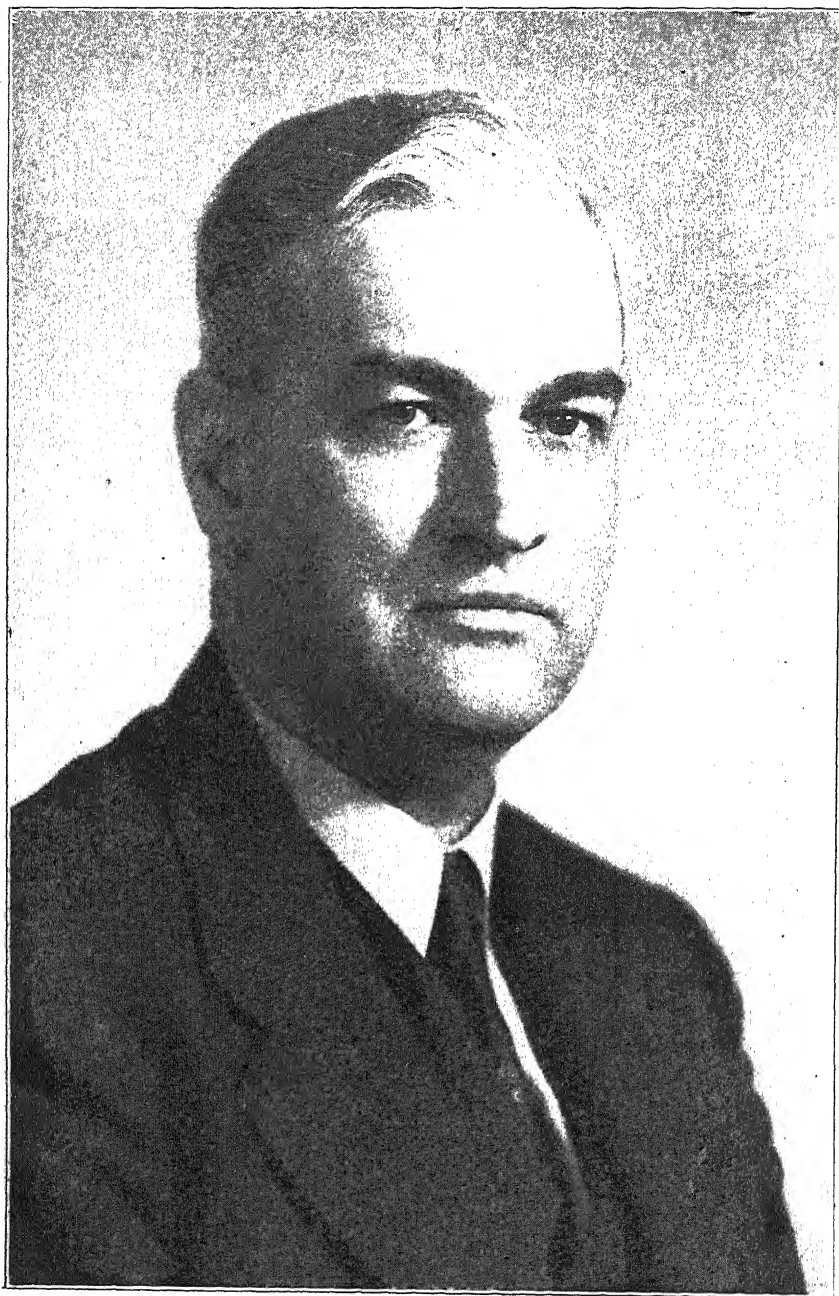
#### REGISTRATION COMMITTEE REPORT

Mr. W. L. Slatter, Chairman of the Registration Committee, reported the registration of members as follows:

Ohio .....	78	New Jersey .....	8	West Virginia .....	3
Illinois .....	52	North Carolina .....	7	California .....	2
New York .....	36	Canada .....	5	Florida .....	2
Wisconsin .....	25	Nebraska .....	5	New Hampshire .....	2
Michigan .....	21	Texas .....	5	Washington .....	2
Washington, D. C. ....	16	Virginia .....	5	Arkansas .....	1
Pennsylvania .....	15	Kentucky .....	4	Delaware .....	1
Missouri .....	12	Oklahoma .....	4	Georgia .....	1
Minnesota .....	11	Tennessee .....	4	Kansas .....	1
Indiana .....	11	Vermont .....	4	Montana .....	1
Iowa .....	11	Connecticut .....	3	Nevada .....	1
Maryland .....	9	Louisiana .....	3	South Carolina .....	1
Massachusetts .....	8	Oregon .....	3	Maine .....	1
TOTAL REGISTRATION .....		384	(38 States and Canada)		
Members .....		324			
Non-Members .....		60			
Banquet Tickets .....		303			

Upon motion duly seconded the report was accepted.

The Secretary-Treasurer then read the minutes of the meetings of the Executive Board which follow. He moved and Mr. Borland seconded that the minutes of the Executive Board be approved and that all actions taken



ARTHUR C. RAGSDALE, *President*

during the past year by the Executive Board be approved by this Association. The motion carried unanimously and the meeting adjourned.

#### INSTALLATION OF OFFICERS

At the annual banquet held at the Southern Hotel on Thursday evening, June 22, President A. C. Dahlberg installed the newly elected officers:

Mr. P. A. Downs of Nebraska and Mr. J. W. Linn of Kansas were installed as Directors for a period of three years in absentia. Mr. J. A. Nelson of Montana was installed as Vice-President and Mr. A. C. Ragsdale of Missouri was duly installed as President of the American Dairy Science Association before the assembly of 303 persons.

#### MEETING OF THE EXECUTIVE BOARD, AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, *Secretary-Treasurer*

*Columbus, Ohio, 2:00 P.M., June 19, 1944*

A meeting of the Executive Board of the American Dairy Science Association was held in Townshend Hall, Monday, June 19, 1944, at 2:00 P.M.

Present: President A. C. Dahlberg, Vice-President A. C. Ragsdale, Secretary-Treasurer R. B. Stoltz, Editor T. S. Sutton, Directors W. E. Petersen, G. M. Trout, R. B. Becker, C. L. Blackman, P. H. Tracy. Guests, E. S. Guthrie and H. F. Judkins. Absent: J. C. Knott and H. P. Davis.

Upon motion duly seconded the minutes of the last Annual Meeting were approved as published in the Proceedings.

Editor Sutton presented the Editor's report, and upon motion by Mr. Petersen and second by Mr. Trout it was accepted.

The Secretary-Treasurer's report was read, and upon motion by Mr. Trout and second by Mr. Tracy it was accepted.

The Auditing Committee's report was read, and upon motion by Mr. Tracy and second by Mr. Petersen, it was accepted and the committee discharged.

The budget for the year 1945, which had been prepared and mailed to the Directors a month previous, was approved on motion by Mr. Petersen and second by Mr. Tracy.

By ballot the Executive Board elected Mr. George Holm a member of the Journal Management Committee for a period of three years beginning at the end of this Annual Meeting.

Mr. Ragsdale moved and Mr. Tracy seconded that the three charter members, Mr. C. C. Hayden, Mr. E. S. Guthrie, and Mr. W. J. Fraser, who have maintained membership since the beginning of the organization be elected to honorary life membership. Mr. O. F. Hunziker, a fourth living charter member, was elected to honorary membership in 1942.

Mr. Trout moved and Mr. Blackman seconded that those men, who upon retirement, have been members of the American Dairy Science Association for a period of 25 years or more immediately before retirement, and who have notified the Secretary of their retirement and eligibility, shall become life members without paying dues upon action of the Board.

Mr. Trout moved and Mr. Blackman seconded that Dean Chris Larsen, Mr. E. G. Hastings and Mr. L. A. Rogers be made life members.

Mr. Petersen moved and Mr. Blackman seconded that the Editor and Secretary-Treasurer be elected annually by the Executive Board and be reelected annually as often as mutually agreeable. The term of office shall begin on January 1 following election. Mr. Ragsdale moved and Mr. Tracy seconded that Mr. Sutton be elected Editor. Mr. Blackman moved and Mr. Trout seconded that Mr. Stoltz be elected Secretary-Treasurer. Mr. Petersen moved and Mr. Trout seconded that the Executive Board express their appreciation to Mr. Sutton and Mr. Stoltz for their excellent services to our Association.

Adjournment.

*Monday, June 19, 1944, 7:30 P.M.*

Present: President Dahlberg, A. C. Ragsdale, T. S. Sutton, C. L. Blackman, R. B. Becker, W. E. Petersen, G. M. Trout, H. P. Davis, R. B. Stoltz. Guest: A. A. Borland. Absent: P. H. Tracy and J. C. Knott.

Mr. Becker moved and Mr. Petersen seconded that the American Dairy Science Association join the Inter-Association Council for Animal Disease and Production, that the Secretary be authorized to pay the dues, and the President appoint a representative from our organization for the ensuing year.

Mr. Davis moved and Mr. Petersen seconded that the action of the Board concerning the following that were taken by mail be validated:

(1) That \$1800 be appropriated to reproduce 13 numbers of back Journals,

(2) That \$2220 be invested in government bonds,

(3) That student affiliates, who have been in the armed service for several years, be given permission to become active members without paying the \$5.00 initiation fee,

(4) That the Secretary be authorized to advertise and pay \$300 for 100 copies of the 26th volume of the JOURNAL OF DAIRY SCIENCE.

It was moved by Mr. Davis and seconded by Mr. Petersen that in view of the suggestion from the National Research Council that the representative of the American Dairy Science Association be chosen from outside of the eastern states. The Board suggests that the President give consideration to the request.

Mr. A. A. Borland, of the Journal Management Committee, gave his report, and upon motion duly seconded it was approved.



Mr. Becker moved and Mr. Davis seconded that the invitation of Iowa State College to hold our annual meeting in 1945 be accepted, and that Wisconsin, Minnesota, Kentucky, and Illinois be thanked for their invitations.

Mr. Petersen moved and Mr. Trout seconded that the suggestion concerning "fellows" be referred to our committee on "Special Recognition" made up of our last three presidents to report at the next annual meeting.

Mr. H. P. Davis, Chairman of the General Program Committee, submitted a statement and recommendation.

After a long discussion, the Directors adjourned without action.

*Tuesday, June 20, 1944, 6:00 P.M.*

A meeting of the Executive Board of the American Dairy Science Association was held at the Faculty Club, Tuesday, June 20, 1944, at 6:00 P.M.

Present: President A. C. Dahlberg, Vice-President A. C. Ragsdale, Secretary-Treasurer R. B. Stoltz, Editor T. S. Sutton, Directors P. H. Tracy, C. L. Blackman, R. B. Becker, W. E. Petersen, G. M. Trout, H. P. Davis. Guest, H. F. Judkins. Absent: J. C. Knott.

Mr. Tracy moved and Mr. Petersen seconded that the minutes of the meetings held on June 19 be approved as corrected.

Mr. Becker moved and Mr. Trout seconded that the President be authorized to represent or appoint a representative to take part in inter-association or governmental activities which affect our industry or profession.

Mr. H. F. Judkins, Chairman of the Committee to Investigate the Training and Employment of Milk Sanitarians, appeared before the Executive Board and gave the report which appears on pages 691-700 of this issue.

Mr. Petersen moved and Mr. Blackman seconded that the report be accepted with thanks.

Mr. Stoltz moved and Mr. Trout seconded that the report be published in the JOURNAL OF DAIRY SCIENCE.

Mr. Petersen moved and Mr. Blackman seconded that a committee consisting of the President, Editor, and Secretary be appointed to negotiate, with power to act relative to the preparation of a 10-Year Index.

Mr. Petersen moved and Mr. Blackman seconded that the request of the Union of American Biological Societies be tabled.

The meeting then adjourned.

*Wednesday, June 21, 1944, 6:00 P.M.*

A meeting of the Executive Board of the American Dairy Science Association was held at the Faculty Club, Wednesday, June 21, 1944, at 6:00 P.M.

Present: President A. C. Dahlberg, Vice-President A. C. Ragsdale, Secretary-Treasurer R. B. Stoltz, Editor T. S. Sutton, Directors P. H. Tracy, C. L. Blackman, R. B. Becker, W. E. Petersen, G. M. Trout, H. P. Davis. Guest, J. A. Nelson. Absent: J. C. Knott.

Mr. Becker moved and Mr. Tracy seconded that the minutes of the Executive Board of June 20 be approved.

Mr. Tracy moved and Mr. Trout seconded that the Chairman of the Committee on post-war problems in dairy manufacturing and distribution be authorized to publish the report in trade papers and to mimeograph it for distribution.

Mr. Petersen moved and Mr. Ragsdale seconded that the Board go on record as commending the Ohio members for the splendid way in which they have handled the convention in every respect.

Mr. Davis moved and Mr. Becker seconded that it be the policy of the Board that the authorization of travel expense be limited to the President, Secretary, and Editor or their representatives. The motion carried not unanimously.

The meeting was then adjourned.

*Thursday, June 22, 1944, 1:00 P.M.*

A meeting of the Executive Board of the American Dairy Science Association was held in Campbell Hall, Thursday, June 22, 1944, at 1:00 P.M.

Present: President Dahlberg, Vice-President Ragsdale, Secretary R. B. Stoltz, Editor Sutton, Directors Blackman, Tracy, Becker, Davis. Guest, J. A. Nelson. Absent: Directors Petersen, Trout, and Knott.

Mr. Becker moved and Mr. Tracy seconded that the minutes of the Executive Board of June 21 be approved.

Mr. Becker moved and Mr. Blackman seconded that the budget be changed so that our Association could take care of travel expenses to Inter-Association affairs by our representatives that are appointed by the President. The budget, will, therefore, contain \$100 for representatives' travel, and the emergency fund will be reduced from \$805 to \$705.

The meeting then adjourned.

AMERICAN DAIRY SCIENCE ASSOCIATION PRESENTED  
BORDEN AWARDS TO P. H. PHILLIPS  
AND A. C. DAHLBERG

*Columbus, Ohio, June 22, 1944*

Mr. C. C. Hayden, of the Ohio Agricultural Experiment Station of Wooster, a charter member of the American Dairy Science Association was toastmaster at the annual Association banquet, and presented Mr. W. E. Krauss, Chairman of the Dairy Production Borden Award Committee, who made the following statement:

"The Dairy Production Award Committee, after careful consideration of the accomplishments of a long list of worthy candidates, has unanimously

selected as the winner of the Borden Award in Dairy Production Dr. Paul H. Phillips of the University of Wisconsin.

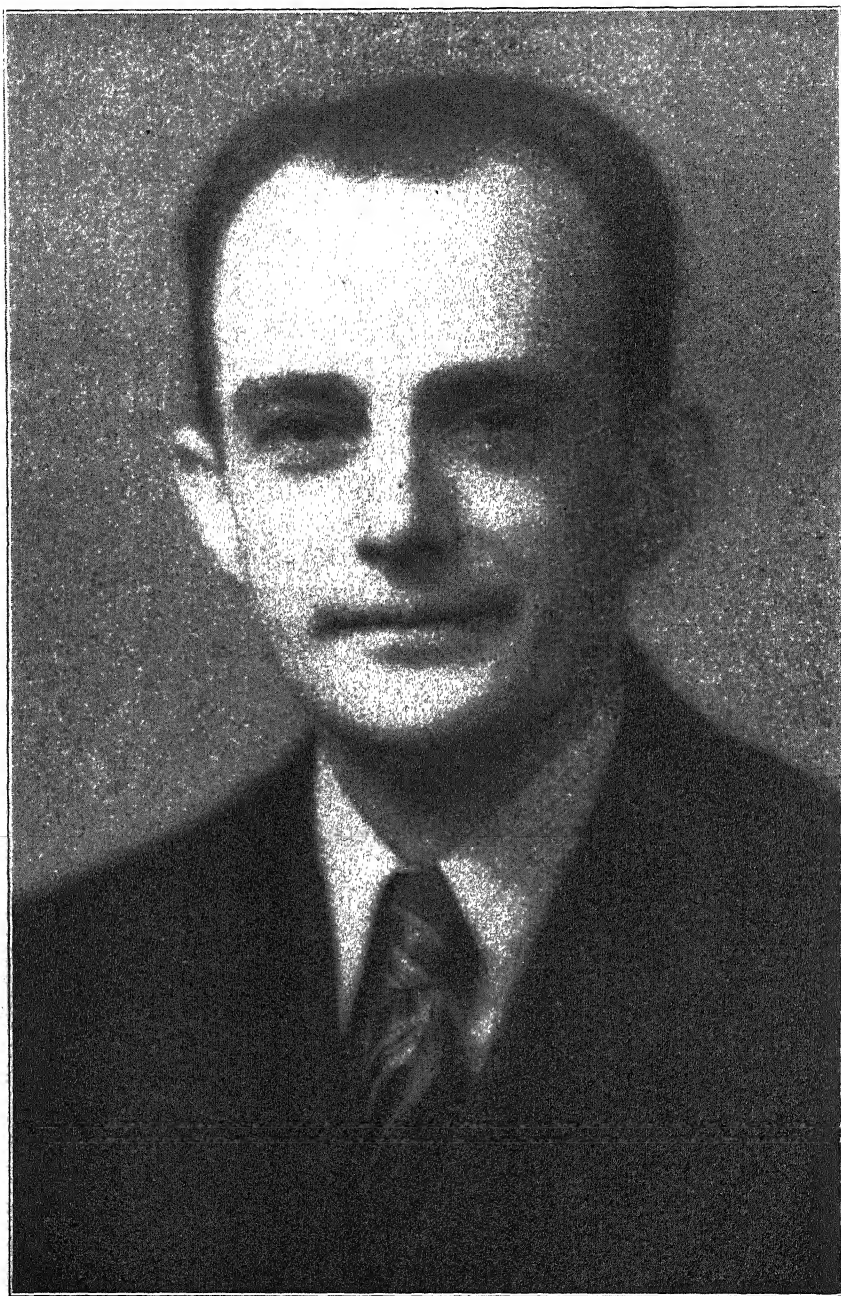
"During the period 1939 through 1943 Dr. Phillips has been senior author or co-author of 38 scientific articles, 23 of which have been directly concerned with dairy production problems or fundamental studies leading to their solution. Most of this work while involving the application of biochemistry, histology, and pathology to fundamental science, has nevertheless had wide practical application and has aided materially in answering questions of fundamental economic importance in the field of dairy production.

"The results of his fluorine studies with dairy cattle, where different levels of this element were fed, have served as a guide in formulating our present recommendations regarding the tolerable limits of fluorine in dairy rations. At a time when the common low-fluorine phosphorus supplement, bone meal, is short in supply and there is a tendency to introduce various types of high fluorine phosphates, the work of Dr. Phillips assumes current as well as fundamental significance.

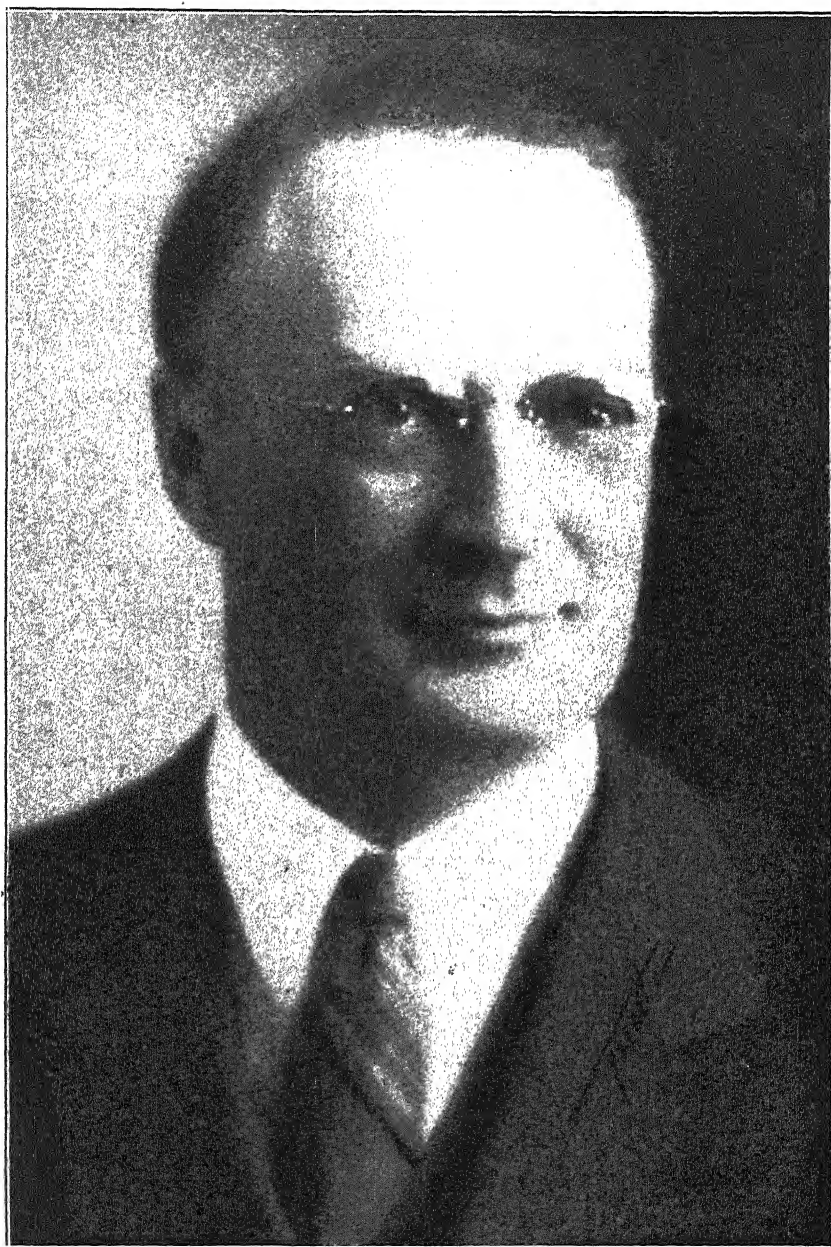
"The greatest contribution made by Dr. Phillips to the field of dairy production, and perhaps the greatest contribution to the livestock industry during the past decade, has been the series of studies concerned with reproduction. These have led to the discovery and widespread adoption of a sperm-preservation technique and a promising treatment for maintaining fertility in both bulls and cows. The far-reaching effects of the egg-yolk-buffer diluent for spermatozoa and the ascorbic acid treatment of sterility are perhaps most appreciated at the present time in artificial breeding associations, but their value filters down even to the individual farmer. Although these original techniques eventually may yield to modified or improved procedures they nevertheless constitute an important milestone in the development of dairy cattle breeding.

"Another series of scientific achievement, in which Dr. Phillips was the prime mover, is that concerned with the nutrition of the young calf. The interrelationships between vitamin A, carotene, ascorbic acid and factors of the B-complex, and the possible role of these factors in reducing calf diseases and mortality, constitute basic information which opens the door to new achievements in a much-neglected field.

"At a time like this there probably is sufficient interest in the background of a man who is recognized publicly as a scientist to warrant a brief biographical sketch. Dr. Phillips was born in Illinois but really grew up on a ranch in Wyoming and received his early schooling there. After graduating from Washington State College in 1923 he spent several years in Russia, where, among other duties, he was assistant director of the Agricultural School in the Caucasus operated by the Near East Relief Foundation. Dr. Phillips entered the graduate school of the University of Wisconsin in 1929



DR. PAUL H. PHILLIPS



DR. ARTHUR C. DAHLBERG

and received the degree of Doctor of Philosophy from that institution in 1933. He now holds a full professorship in the Department of Biochemistry.

"An animal husbandman at heart, a biochemist by training, and at present a dairyman after hours, Dr. Phillips has combined fortunate traits of character, training and experience in doggedly achieving his present scientific status. A good teacher but a hard taskmaster, he has helped in the development of many students, and a corps of graduate students who have been associated with him in the work that was done and in the authorship of the papers that constituted the basis for the committee's selection."

Dr. Phillips came to the speakers' table and Mr. Wentworth of the Borden Company stated: "I really believe, Dr. Phillips, that that is the longest record of achievement I have ever heard in regard to any person chosen to receive this Award. I think you are to be congratulated upon it. I was also interested to note that the letter "W" is connected with your life—Wyoming, Washington, and Wisconsin. I don't know that it's anything significant, but I've lived under the letter "W" for a long time. However, it does give me a great deal of pleasure on behalf of the Borden Company to present the Borden Award, this medal that I trust you can keep forever, and \$1000 as a partial measure of our appreciation for your achievements. I'm going to whisper this so that all can hear. You don't need to report this on your income tax."

Dr. Paul Sharp, Chairman of the Borden Award Committee for Manufacturing, was then introduced. Dr. Sharp then made the following statement:

"The recipient of the Borden Award in Dairy Manufacturing for this year received his Bachelor's and Master's degrees from the University of Minnesota and his Doctor's degree from Illinois. He is a practical dairy scientist who has made notable contributions to our knowledge of the viscosity of cream, cream whipping, the texture of ice cream, the use of gelatin in ice cream and water ices, the sweetness of sugars and their use in ice cream, studies on high-temperature short-time pasteurization, and a method for the manufacture of cream and Neufchatel cheese. He has also carried out investigations in the field of production such as the influence of the rate of milking upon yield and of the temperature on the filtering of milk.

"It was the unanimous opinion of the Borden Award Committee in Dairy Manufactures of the American Dairy Science Association that Dr. A. C. Dahlberg be selected for the 1944 research award in Dairy Manufactures.

"From 1921 to 1942 Dr. Dahlberg was Chief of Research in dairying at the New York Experiment Station in Geneva.

"Dr. Dahlberg has been active in association affairs as follows:

Editor of the JOURNAL OF DAIRY SCIENCE—1928 to 1938

Edited abstracts of the International Association of Ice Cream Manufacturers—1927 to 1937

Edited abstracts of the International Association of Milk Dealers—1930 to 1937

Delegate to the World's Dairy Congress at Berlin—1937

President of the New York Dairymen's Association—1926 to 1927

President of the New York Jersey Cattle Club—1926 to 1928

Now President of the American Dairy Science Association

“Mr. Wentworth, I am pleased to present to you Dr. Dahlberg the outgoing President of the American Dairy Science Association.”

Mr. Wentworth then replied as follows:

“You remember that I received a letter last December from you suggesting that there be some changes in the rules under which this Award was given. That is a fact. One of the things which he undertook to do while President of this Association was to effect a change in the regulations of the management of this Association of these Awards. If he had been successful in making those changes, I would say that he had been a successful officer. But his success comes to him for other reasons as has been indicated by Dr. Sharp. Of course, Dr. Sharp lived up there in the State of New York for so long that he can just get up here and recite Dr. Dahlberg's accomplishments in contrast to Dr. Krauss reading Dr. Phillips' from a paper. Anyway, it's a great pleasure for me on behalf of the Borden Company to present to you this Award for 1944 in Dairy Manufacturing work; first, as usual, is the medal, and second is the check for \$1,000.”





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## A BREED COMPARISON IN THE VITAMIN D CONTENT OF MILK WITH NOTES ON A MODIFIED TECHNIQUE FOR THE VITAMIN D ASSAY OF LOW-POTENCY FATS AND OILS\*

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Information on breed differences in the vitamin D content of milk is decidedly meager. Bechtel and Hoppert (1) have reported an extensive study of seasonal variations of vitamin D in normal cow's milk. Holsteins and Guernseys were included in this study so some comparisons are possible between these two breeds. Normal herd management was employed so no information is available as to the actual vitamin D intake. Under these conditions the authors noted that there was little difference in the anti-rachitic potency of the milk fat of the two breeds. They pointed out that the higher percentage of fat in the Guernsey milk largely accounted for the higher vitamin D potency per quart of milk for this breed. The vitamin D values for the Guernsey milk varied from 4.8 to 43.8 U.S.P. units per quart whereas the extreme values for the Holstein milk were 3.1 to 27.7 U.S.P. units per quart. Kon and Henry (5) have reported a comparison between Guernsey and Shorthorn cows under pasture conditions with supplemental feeds as required. Under these conditions the actual intake could not be measured. The Guernsey butterfat contained 0.35 International Units per gram and the Shorthorn 0.28. The difference was not statistically significant so these authors also suggested that the vitamin D activity of the milk of the two breeds is in proportion to the fat percentage of the milk.

On the other hand, the recommendations for feeding irradiated yeast to dairy cows for the production of vitamin D milk with a uniform potency per quart called for a given amount of yeast for a given level of milk production (7). These recommendations were based on extensive research which seemed to indicate that differences in breed, fat percentage of the milk, and such factors were relatively insignificant. It is quite evident that if milk of varying butterfat content is to have the same vitamin D potency

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per quart there must be a compensating difference in the potency of each gram of fat in the milk. This relationship differs somewhat from the results of the investigations (1) and (5) already reviewed. Of course, these differences may result in part from the fact that normal herd management was used in one case while comparatively large supplements of vitamin D were used in the other. The experiments being reported in this paper were designed to gain more information on this problem and to study breed differences in the vitamin D content of milk using normal rations to supply constant, known amounts of vitamin D.

#### EXPERIMENTAL METHODS

*Plan of main experiment.* Holsteins and Jerseys were used in this experiment, thus providing a wide difference in the quantity and butterfat percentage of the milk produced. Animals, representative of the breeds concerned were carefully paired to eliminate as many variables as possible. Particular attention was given to have freshening dates close together for each pair so that seasonal and management differences would not affect the vitamin D values.

Three pairs of cows have been studied for one complete lactation. An attempt was made to equalize the vitamin D reserves of each pair by either placing them on a vitamin-D-deficient ration for a preliminary period of two months or by feeding the experimental ration with a known vitamin D content for two or three months before freshening. Alfalfa hay furnished the only source of vitamin D. The cows were exercised after dark or in a shed. Sufficient hay was secured at the beginning to feed each pair throughout the entire lactation. The vitamin D intake was maintained at a constant level by feeding the same amount of alfalfa hay to each cow for the duration of the lactation. The largest amount of hay that the Jersey cow would eat regularly determined the level of hay feeding for each pair. Molasses beet pulp was used to supply additional bulk to the Holstein. Vitamin D determinations were made on the alfalfa hay two or three times during the lactation period. A three-day composite sample of milk was saved each month for determining the butterfat percentage in the milk and securing a sample of pure butterfat for vitamin D assay. The milk was separated, the cream churned, and the butter melted and centrifuged to obtain the butterfat for vitamin D testing. The butterfat samples were kept frozen in sealed jars until assayed.

Because of the large amount of work involved in making the vitamin D assays it was necessary to run one pair of cows at a time. When the third pair was run blood plasma samples were taken simultaneously with the butterfat samples to assist in explaining some of the earlier observations. The regular line-test technique was used in making the vitamin D determinations on the alfalfa hay, blood plasma, and the butter-fat from the first

two pairs of cows. A modified method was used for the butterfat of the third pair to secure greater accuracy.

*The modified method for vitamin D assay of low potency fats and oils.* Since natural feeding stuffs were being used as the source of vitamin D in this experiment it was expected that the potency of the butterfat would be comparatively low. This raised the problem of how best to make the vitamin D determinations as rats will consume only limited amounts of the fat to be tested during the first 8 days of the usual 10-day test period. The alcoholic extraction method reported by Bechtel and Hoppert (1) was first tried but it was found that only 50-75 per cent of the vitamin D was being recovered. Further tests with modifications of this method also proved ineffective. Since Kon and Booth (3, 4) had earlier found difficulty in recovering vitamin D from butterfat either in the non-saponifiable portion or by alcoholic extraction it was decided to assay the fat direct rather than to attempt concentrating it. This method was employed for the first two pairs of cows but by that time Gridgeman, Lees, and Wilkinson (2) had reported encouraging results from adjusting the mineral content of the test diet to insure greater accuracy in the testing of low potency fats for vitamin D.

Gridgeman *et al.* (2) found a greater healing response to 0.5 International Unit of vitamin D daily with rats receiving a rachitogenic diet to which 38 per cent of an inert cooking fat had been added than in litter mates receiving the rachitogenic diet only. Their work also showed that healing responses were inversely proportional to the daily intake of salt mixture and were not related to variations in the phosphorus, protein, and fat intake. The salt mixture contained 75 per cent of  $\text{CaCO}_3$ , 15 per cent of  $\text{NaCl}$ , and traces of other minerals but no phosphorus. Thus the addition of fats or oils to the rachitogenic diet lowered the daily intake of salt mixture, by diluting the mineral content of the diet and by decreasing the daily consumption because of the increased caloric value of the diet. If adjustments in the salt-mixture content of the diets were made so that the intake on test diets containing fat compared favorably with the intake on the rachitogenic diet alone, the healing responses to a given amount of vitamin D were more nearly equal. With these principles in mind, studies were made of adjustments in the mineral content of rachitogenic diets used for testing low potency fats and oils for vitamin D.

In our laboratory 12 grams of butterfat had been mixed with 28 grams of the Steenbock rachitogenic diet No. 2965 to make a total of 40 grams of diet. This was consumed in about the first 8 days of the test period. The regular rachitogenic diet was then fed for the remainder of the 10-day test period. Inspection of our records showed that litter-mate rats receiving the rachitogenic diet plus the International Vitamin D Standard consumed 55 grams during the first 8 days. Adjustments were then made so that

40 grams of test diet containing the fat had the same salt-mixture content as the 55 grams of regular rachitogenic diet. On this basis each test diet was made up as follows:

17.80 grams yellow corn meal
12.00 grams fat or oil
8.00 grams wheat gluten
1.65 grams $\text{CaCO}_3$
0.55 grams NaCl
<hr/> 40.00 grams

For purposes of discussion this modified diet will be referred to as the mineral-adjusted diet to differentiate it from the regular rachitogenic diet. Tests were first made to determine how much healing, if any, would be initiated by feeding 12 grams of an inert fat or oil using the regular line test procedure the same as though a vitamin D assay were being made. The mineral-adjusted diet was compared with the regular rachitogenic diet and with the healing given by 5 International Units of vitamin D Standard. Ten litter-mate rats were used for each comparison. Studies were made using a sample of butterfat from a vitamin-D-deficient cow, a cooking fat, olive oil, and a cooking oil. The results are shown in table 1.

TABLE 1

*A comparison of the healing responses to 12 grams of inert fats and oils fed with regular rickets diet and with a mineral-adjusted diet using the regular line-test technique for vitamin D assays*

Fat or oil tested	Healing responses with different diets		
	Rachitogenic only plus 5 International Units of vitamin D	Regular rachitogenic diet plus 12 grams of fat	Mineral-adjusted diet plus 12 grams of fat
Butterfat* .....	1.15†	0.07	0.06
Cooking fat .....	1.93	0.86	0.14
Olive oil .....	0.72	0.26	0.03
Cooking oil .....	1.14	0.10	0.06

\* This butterfat sample was from a vitamin-D-deficient cow and contained little or no vitamin D.

† A narrow, continuous line of healing is given a value of 1.0.

It can be seen from table 1 that there was some healing response with all of the supposedly inert materials tested even with the mineral-adjusted diet but it was considerably higher when these same fats and oils were fed with the regular rachitogenic diet. The healing responses obtained from 5 International Units of vitamin D Standard gives a basis for evaluating the healing initiated by the fats alone.

In a further study, 5 International Units of vitamin D Standard were fed to ten litter-mate rats receiving 12 grams of the inert fat plus 28 grams of either the regular rachitogenic diet or the mineral-adjusted diet. These

TABLE 2

*The apparent value of 5 International Units of vitamin D Standard when fed with diets containing 12 grams of inert fat mixed with regular rachitogenic diets and with mineral-adjusted diets using the line-test technique*

Fat or oil tested	Apparent value of 5 I.U.† of vitamin D when fed with	
	12 grams of fat plus regular rachitogenic diet	12 grams of fat plus mineral-adjusted diet
Butterfat* .....	6.06	5.00
Cooking fat .....	7.35	5.74
Olive oil .....	5.41	4.10
Cooking oil .....	5.57	4.72
Average .....	6.10	4.89

\* This butterfat sample was from a vitamin-D-deficient cow and contained little or no vitamin D.

† I.U. = International Unit of vitamin D.

healing responses were evaluated against litter-mate rats receiving 5 International Units of vitamin D reference oil as is used in making a regular vitamin D assay. The purpose was to determine how much, if any, the value of a known amount of vitamin D was enhanced by feeding it with the fat-containing diets. The results are shown in table 2.

It can be seen from table 2 that the apparent value of 5 International Units of vitamin D was increased in every case when fed with an inert fat added to the regular rachitogenic diet. When the mineral-adjusted diet was used the apparent value was sometimes higher than the 5 units actually fed and sometimes lower with an average of just slightly less than 5.

One other piece of evidence was obtained which gave further support to the superiority of the mineral-adjusted diet under the conditions of these determinations. Vitamin D assays were made on ten samples of butterfat using both the mineral-adjusted diet and the regular rachitogenic diet. The usual line-test technique was followed. Litter-mate rats were given 2 units

TABLE 3

*A comparison of the vitamin D in ten samples of butterfat as determined by using the regular rachitogenic diet and the mineral-adjusted diet*

Number of butterfat sample	I.U.* of vitamin D per gram of butterfat			
	Cow 13E		Cow 150	
	Rachitogenic diet	Mineral-adjusted diet	Rachitogenic diet	Mineral-adjusted diet
1	0.33	0.23	0.68	0.35
2	0.40	0.25	0.50	0.30
3	0.22	0.19	0.37	0.32
4	0.22	0.20	0.38	0.31
5	0.27	0.20	0.28	0.22
Average .....	0.29	0.21	0.44	0.30

\* I.U. = International Unit of vitamin D.

and 5 units, respectively, of International Standard vitamin D and the final potencies calculated by the use of a master curve of response to graded doses of vitamin D. The results are shown in table 3.

In every case the value was higher with the regular rachitogenic diet than when the mineral-adjusted diet was employed. The average difference was 38 per cent higher for one cow and 47 for the other. With more potent butterfat the percentage differences would probably not be so great.

While we still do not have a satisfactory method for concentrating the vitamin D of low potency butterfat samples, the combined evidence from the work just presented indicates that the mineral-adjusted diet as used under the conditions of this experiment gave more accurate results than where the regular rachitogenic diet was employed. Consequently the vitamin D values for the butterfat were determined for the third pair of cows in this experiment by using the mineral-adjusted diet technique. Just what adjustments would be necessary for different doses of butterfat require further investigation.

#### RESULTS AND DISCUSSION

The pertinent data from the first pair of cows are shown in table 4.

These two animals were typical of their respective breeds. The Holstein gave between two and three times as much milk as the Jersey but the butterfat percentage was only about half as high. As a result the total butterfat production was slightly higher for the Holstein. Each cow received 19,000 International Units of vitamin D daily throughout the lactation in the alfalfa hay fed. Under these conditions the butterfat from the Jersey averaged about 50 per cent higher in International Units of vitamin D per gram. The higher butterfat content of Jersey milk and the higher vitamin D potency per gram made an average of approximately 30 International Units of vitamin D per quart of milk whereas the Holstein milk averaged only about 10 units per quart. Because of the higher milk production of the Holstein the total amount of vitamin D recovered daily in the milk was not greatly different for the two cows. The recovery for the two cows amounted to 1.50 to 1.75 per cent of the intake during the flush of the lactation and declined to 0.50 to 0.75 per cent toward the end. The percentage of recovery of ingested vitamin D in the butterfat, and the greater efficiency of recovery at higher levels of production, agree in general with the findings of other investigators. It is interesting to note that the vitamin D potency per gram of butterfat decreased with the decrease in milk production near the end of the lactation but the percentage of butterfat in the milk increased proportionately, so the potency per quart of milk remained quite constant throughout the lactation.

In order to conserve space the data for the second pair of cows will not be published. The general relationships were essentially the same as for

the first pair. The alfalfa hay fed to the second pair contained less vitamin D so each cow received only 6,500 International Units daily. This resulted in a lower vitamin D potency per gram of butterfat and per quart of milk. The Holstein milk averaged about 6 International Units per quart and the Jersey about 15.

TABLE 4

*A comparison of the vitamin D content of the milk and butterfat produced by a Jersey and a Holstein cow receiving 19,000 International Units of vitamin D daily*

First pair

Month of lactation	Daily production			Vitamin D			
	Milk, lbs.	Fat, %	Fat, gms.	I.U.* per gm. of fat	I.U. per qt. of milk	Recovered in fat	
						Daily I.U.	% of intake
Holstein cow—390							
I .....	45.6	3.85	793.8	0.31	11.6	246	1.09
II .....	55.5	3.82	961.6	0.37	13.8	356	1.86
III .....	58.0	3.17	830.1	0.40	12.3	332	1.74
IV .....	54.7	2.81	698.5	0.43	11.8	300	1.75
V .....	41.9	2.06	390.1	0.45	9.0	176	1.06
VI .....	32.2	3.77	548.9	0.29	10.6	159	0.90
VII .....	32.4	3.60	526.2	0.25	8.8	132	0.69
VIII .....	32.4	3.30	485.4	0.25	8.0	121	0.63
IX .....	32.1	3.35	485.4	0.27	8.8	131	0.69
X .....	35.4	3.20	512.6	0.23	7.2	118	0.62
XI .....	33.7	3.49	535.2	0.27	9.2	145	0.76
XII .....	28.2	3.95	503.5	0.26	10.0	131	0.69
XIII .....	15.5	4.27	299.4	0.30	12.5	90	0.47
Jersey cow—146							
I .....	17.0	5.81	449.1	0.28	15.9	126	0.60
II .....	16.0	6.09	444.5	0.53	31.6	236	1.23
III .....	16.8	5.40	412.8	0.61	32.3	252	1.43
IV .....	15.6	5.19	367.4	0.63	31.8	231	1.47
V .....	15.3	5.45	376.5	0.63	33.3	237	1.40
VI .....	13.3	6.11	367.4	0.42	24.9	154	0.83
VII .....	12.9	6.40	376.5	0.43	27.0	162	0.85
VIII .....	12.1	6.60	358.3	0.49	31.3	176	0.92
IX .....	7.8	7.20	254.0	0.48	33.6	122	0.64
X .....	7.4	7.10	235.9	0.44	30.3	104	0.55
XI .....	6.3	8.00	226.8	0.43	33.6	98	0.51

\* I.U. = International Unit of vitamin D.

For the third pair of cows the vitamin D content of the blood plasma was determined simultaneously with the monthly butterfat samples and the mineral-adjusted diet was used for the vitamin D determinations on the butterfat. The data for the third pair of cows are shown in table 5.

The general relationships discussed for the first pair of cows also hold true for the data from the third pair. Although these cows received 24,200 International Units of vitamin D daily which is slightly more than the first pair the potency of the butterfat per gram is slightly less. The fact that

these assays were made with the mineral-adjusted diet which gives somewhat lower but more accurate results undoubtedly explains this difference. Again, the Jersey butterfat has a higher vitamin D potency per gram than the Holstein. The potency of the blood plasma is also higher for the Jersey even though the vitamin D intake was kept the same for both cows starting at two months before calving. The higher potency of blood plasma undoubtedly explains why the Jersey butterfat had a higher vitamin D potency

TABLE 5

*A comparison of the vitamin D content of the blood plasma and of the milk and butterfat produced by a Jersey and a Holstein cow receiving 24,200 International Units of vitamin D daily*  
Third pair

Month of lac- tation	Daily production			Vitamin D				
	Milk, lbs.	Butter- fat, %	Butter- fat, gms.	I.U.† per ml. of blood plasma	I.U. per gm. of but- terfat	I.U. per qt. of milk	Recovered in fat	
							Daily I.U.	% of intake
Holstein Cow—13E*								
I	48.8	3.9	861.0	2.33	0.24	9.12	206.8	0.855
II	41.0	1.6	299.4	2.53	0.25	3.91	74.9	0.310
III	39.8	3.8	685.0	3.34	0.19	7.03	130.2	0.538
IV	37.0	4.0	671.0	3.21	0.20	7.78	134.2	0.555
V	38.3	3.6	626.0	3.57	0.20	7.01	125.2	0.517
VI	32.8	3.8	567.0	3.08	0.19	7.05	107.7	0.445
VII	36.0	3.4	553.4	2.49	0.20	6.60	110.7	0.457
Jersey cow—150								
I	20.2	5.3	485.4	3.71	0.42	21.69	203.9	0.843
II	18.3	5.7	473.1	3.47	0.30	16.66	141.9	0.586
III	20.8	5.7	537.5	4.70	0.32	17.76	172.0	0.711
IV	16.2	6.0	440.9	3.93	0.31	18.15	136.7	0.565
V	19.7	5.6	500.3	4.39	0.22	12.02	110.1	0.455
VI	18.8	6.0	511.7	3.58	0.29	16.96	148.4	0.613
VII	18.0	6.2	506.2	2.25	0.21	12.70	106.3	0.439
VIII	18.8	5.6	477.6	3.00	0.23	12.56	109.8	0.454
IX	18.0	6.2	506.2	2.87	0.23	13.91	116.4	0.481
X	16.3	6.4	473.1	2.85	0.24	14.96	113.5	0.469

\* Lactation incomplete—cow sold as T.B. reactor at end of seventh month.

† I.U. International Unit of vitamin D.

than the Holstein in each of the three pairs of cows used in this experiment. Just why the blood plasma of the Jersey should be higher on the same intake of vitamin D still remains unexplained.

Light, Wilson, and Frey (6) found a direct correlation between the concentration of vitamin D in the blood and the rate of secretion into the milk when cows were fed irradiated yeast. The results of the present investigation show that this same relationship exists when natural feeds alone furnish a much smaller intake of vitamin D. Higher blood plasma potencies are



associated with higher butterfat potencies and lower blood plasma values with lower butterfat values.

It should be noted that in this experiment the Holstein and Jersey cow making up each pair were given the same vitamin D intake. The relationships found will properly apply only where these conditions prevail. Under normal herd management Holstein cows will consume more roughage and hence have a higher vitamin D intake than Jerseys. This would undoubtedly increase the vitamin D potency of the butterfat produced by the Holstein, making it more nearly that of the Jersey. Just what these relationships would be cannot be told without further experimentation. It would also require further investigation to ascertain the breed effect of exposure to sunshine which is ordinarily experienced in normal herd management.

#### SUMMARY

Breed differences in the vitamin D content of milk and butterfat for Holstein and Jersey cows receiving the same vitamin D intake have been investigated. Three comparable pairs of animals have been studied for complete lactation periods. The first pair received 19,000 International Units of vitamin D daily in the alfalfa hay which was the only source of this factor. The second pair received 6,500 units daily and the third pair 24,200. The milk from each cow was saved for a three-day period once each month for determining the percentage of butterfat in the milk and to secure a sample of pure butterfat for vitamin D determination. Blood plasma samples for vitamin D determinations were taken simultaneously with the butterfat samples for the third pair of cows.

The results from all three pairs are in general agreement in showing that under the conditions of this experiment the Jersey butterfat contained somewhat more vitamin D per gram of fat than the Holstein. The higher butterfat percentage for the Jersey milk combined with a higher vitamin D potency per gram of fat made the Jersey milk two to three times more potent than the Holstein milk in vitamin D per quart. The total amount recovered in the butterfat daily was approximately the same for both breeds, however, because of the larger milk production of the Holstein. About 1.50 per cent of the ingested vitamin D was recovered in the butterfat during the flush of the lactation but only about 0.50 per cent near the end of the lactation. The vitamin D potency per gram of butterfat decreased for each breed as the lactation progressed but the butterfat percentage increased proportionately so the vitamin D content per quart of milk remained quite uniform for each cow throughout the lactation period. Data from the third pair of cows indicated that the vitamin D content of the blood plasma was higher for the Jersey. This correlated with the higher potency of the butterfat.

A modification in the mineral content of the rachitogenic diet used for making vitamin D assays has been developed which under the conditions of

this experiment gave lower but more accurate values for the vitamin D content of low-potency butterfat samples.

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# LACTIC ACID IN DAIRY PRODUCTS. I. APPLICATION OF THE HILLIG METHOD\*

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Quantitative determinations of lactic acid and lactates in milk and milk products as means of ascertaining the extent of microbiological spoilage have long been of interest and various methods have been proposed for such analyses. Recently, an intensification of interest in this question has resulted because of the use of lactic acid values by Federal regulatory officials as a basis for judging quality in concentrated and dried milk.

At the present time there are two rather generally accepted quantitative methods for measuring lactic acid in milk: (a) The Troy-Sharp aldehyde procedure (7) and (b) the Hillig colorimetric procedure (1).

The aldehyde procedure involves the precipitation of the interfering substances with copper hydroxide at 45° C., oxidizing the filtrate under carefully controlled conditions with sulfuric acid, manganese sulfate and potassium permanganate, distilling the acetaldehyde produced into sodium bisulfite, and titrating the bound sulfite with iodine. The result is then corrected for the blank on fresh milk and for the lactic acid retained by the precipitate. This method was found by Troy and Sharp to be accurate down to 0.002 per cent of lactic acid in the original milk and to give excellent agreement in the hands of various operators when applied to dried milk.

The colorimetric method utilizes the following steps: (a) Preparation of a milk serum by treatment of the milk with sulfuric and phosphotungstic acids, (b) extraction of the serum with ethyl ether, (c) neutralization of the extract with barium hydroxide and the subsequent precipitation and removal of the alcohol-insoluble barium salts, (d) treatment of the filtrate with carbon and hydrochloric acid, (e) photometric measurement of the intensity of the yellow color produced upon the addition of ferric chloride.

The accuracy of the colorimetric method was determined by Hillig (1) by adding lithium lactate to reconstituted dried skim milk at concentrations varying from 0.002 to 0.150 per cent. Recovery was usually within one milligram per cent.

Limited comparisons of the aldehyde and colorimetric methods have been made by Hillig (2). On two samples of dried skim milk which were sent to collaborators, the Troy-Sharp procedure gave fairly satisfactory results, but results by the colorimetric method were unsatisfactory. The failure of the colorimetric procedure to give uniform results was believed to be due to

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inaccurate standard curves. In another study, Hillig (3) found good agreement between the two methods when they were applied to non-neutralized dry milk, but the aldehyde method gave somewhat lower results on neutralized samples. In more recent work, Hillig (5) proposes an improvement in the procedure for establishing the standard curve for the colorimetric determination which presumably insures greater uniformity in the application of the method.

Until quite recently, the aldehyde technique has been more widely used than the colorimetric method, probably because the former was perfected at an earlier date and more is known concerning the factors which influence the reliability of its results. Troy and Sharp (7) found that titers obtained by the aldehyde method were markedly increased by lipolysis (due to the presence of glycerol), and by such preservatives as formalin and acetone. Slight increases were produced by the use of phenol and salicylic acid, whereas potassium dichromate was reported to decrease the lactic acid recovery. Ethyl and methyl alcohol in amounts of one to two per cent did not interfere with the determination. Products high in sucrose could not be successfully analyzed by the aldehyde method.

These workers also reported mercuric chloride in concentrations of 0.2–0.5 per cent to be a satisfactory preservative for as long as a month for milk kept in dark cool surroundings. Later observations (8) revealed increases in the lactic acid of milk stored in diffused sunlight and preserved with mercuric chloride, increases which were attributed to proteolysis and the formation of degradation products which interfered with the acetaldehyde titration. Heating of the samples to 80° C. for five minutes decreased but did not stop the proteolysis.

At the present time the colorimetric procedure is receiving considerable attention since this technique is being used by the Federal regulatory officials in the examination of milk and other food products. However, information is limited relative to the factors which may influence the reliability of the results secured by this procedure. It was for the purpose of securing such information that the study herein reported was conducted.

#### PROCEDURE

Lactic acid was measured by the Hillig colorimetric method (1) with slight modifications. The calcium lactate-barium hydroxide procedure was used for the standard curve (5).

Colorimetric measurements were made by means of a Cenco-Sheard Spectrophotometer using tubular cells, a wave-length setting of 400 millimicrons, and a blue filter over the entrance slit to reduce stray light. The use of this wave length was established from a transmission curve developed by using the Spectrophotometer and an aqueous solution containing approximately 12 mg. of lactic acid (fig. 1). The curve is similar to that

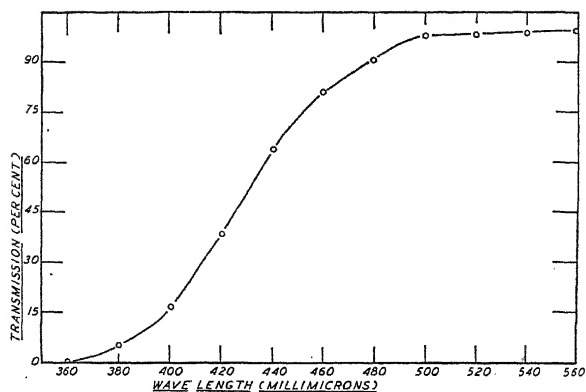


FIG. 1. Transmission curve for a solution of known lactic acid content.

reported by Hillig (1) but indicates somewhat more absorption at the lower wave length values. At 400 millimicrons wave length, the transmission is approximately 19 per cent, which corresponds to approximately the result found by Hillig at 460 millimicrons wave length.

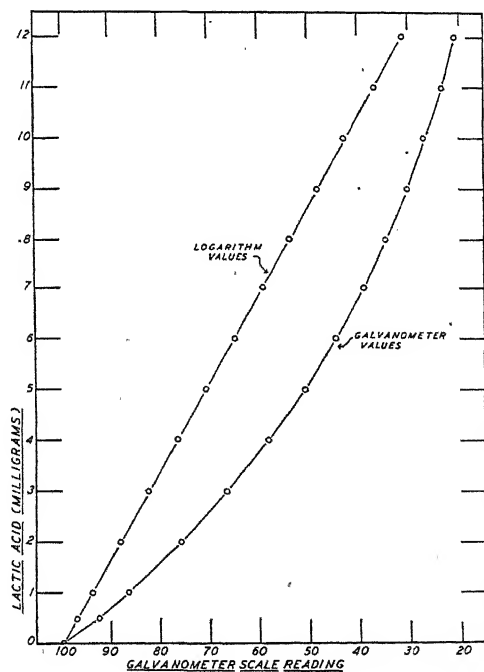


FIG. 2. Standard curve from prepared solutions of known lactic acid content. (Lactic acid concentration plotted directly against galvanometer readings and also against the logarithms of the galvanometer readings. For convenience, the characteristic of the logarithm, i.e., 1 or 2, has been omitted.)

A standard curve was prepared according to the direction of Hillig (5). Curves based both on the direct galvanometer readings and on the logarithm of the galvanometer readings are presented in figure 2. The logarithm values exhibit almost a straight line relationship with the lactic acid concentration, and for this reason were used throughout this study for determining the lactic acid concentrations of the unknowns.

Precautions suggested by Hillig (1), to prevent fading of the color following the addition of the ferric chloride were found unnecessary. Samples were treated with ferric chloride and the color determinations made within 2-3 minutes thereafter. Numerous trials indicated that exposure to diffused daylight for approximately 10 minutes or longer was necessary before any detectable color fading occurred.

Special precautions were found to be necessary to insure properly purified ether for the extraction. Ether of U.S.P. grade was found to vary in its ability to extract lactic acid, but in general, was highly inefficient. In some trials, such ether recovered only approximately 30 per cent of the lactic acid, and frequently less than 50 per cent of the acid was recovered. Satisfactory results were secured when the ether was shaken vigorously with water (four parts ether to one part ordinary tap water) and then distilled just before use. Attempts to substitute Skellysolve A (a petroleum product with a boiling point similar to ether) as the extractant were unsuccessful.

#### RESULTS

*Recovery of added acid.* The accuracy of the Hillig method was ascertained by analyzing milk to which different concentrations of lactic acid had been added. The results of one series of such trials are shown in table 1.

These data indicate that the method possesses a high degree of accuracy and reliability. Recovery of acid was within 1 mg. for all but one sample and the percentage recovery was usually well within 5 per cent. The average recovery for the 14 analyses was 99.7 per cent.

*Lactic acid values of fresh milk.* Milk samples secured from the afternoon milking of cows of the Michigan State College herd were immediately placed in ice water, transported to the laboratory and analyzed for lactic acid by the Hillig technique. The results, presented in table 2, reveal a range in lactic acid from 0.92 mg. to 3.02 mg. per 100 gms. for the six samples, with an average of 1.93 mg. per 100 gms. These are similar to the results given for fresh milk by Troy and Sharp (6) and for commercial, retail samples by Hillig (1). In general, it appears that the lactic acid content of fresh milk, determined by this method, will usually not exceed 3 mg. per cent, but factors of season, disease and stage of lactation or gestation may influence this result.

Even though the data in table 2 indicate the presence of small amounts of lactic acid in strictly fresh milk, it should be realized that these results

TABLE 1

*Recovery of known quantities of lactic acid added to milk*

Sample No.	Duplicate	Acid added	Acid recovered	Difference	Recovery
		<i>mg./100 gm.</i>	<i>mg./100 gm.</i>	<i>mg./100 gm.</i>	<i>per cent</i>
1	a	1.79	1.55	-0.24	86.6
	b	1.79	1.78	-0.01	99.4
2	a	8.97	9.78	+0.81	109.0
	b	8.12	8.58	+0.46	105.7
3	a	17.95	18.07	+0.12	100.7
	b	17.95	17.43	-0.52	97.1
4	a	35.36	34.54	-0.82	97.7
	b	35.90	35.40	-0.50	98.6
5	a	53.04	52.32	-0.72	98.6
	b	53.04	52.44	-0.60	98.9
6	a	70.72	71.68	+0.96	101.4
	b	70.72	72.98	+2.26	103.2
7	a	106.08	105.69	-0.39	99.6
	b	106.08	105.13	-0.95	99.1

may be due to constituents other than lactic acid. In fact, for the present, it is probably more logical to accept these results as representing the residual or blank values of fresh milk rather than as indicating lactic acid. Furthermore, since results obtained by the Hillig (or similar) method are due either entirely or partly to this residual factor (unless a blank correction is made for the milk), they may not, in a strict sense, represent the true lactic acid content. On this basis, therefore, it appears logical to refer to the results of such an analysis as "lactic acid values." This practice is adhered to throughout this study.

*Preservatives.* To determine their influence on the lactic acid values, the following preservatives were added to milk: Mercuric chloride (commercial corrosive sublimate), formalin, potassium dichromate, boric acid, sodium benzoate and phenol.

Following the addition of the preservative, each sample was held at 37° C. for several hours until its control sample was strongly sour to the taste. Lactic acid was determined on the milk immediately following the addition of the preservative and again at the close of the storage period.

The results of this experiment, presented in table 3, reveal that with the exception of potassium dichromate and sodium benzoate, the lactic value

TABLE 2

*Lactic acid values of fresh milk as determined by the Hillig procedure*

Cow No.	Breed	Lactic acid content
		<i>mg./100 gm.</i>
9	Guernsey	1.6
22	Guernsey	2.0
114	Jersey	2.9
274	Holstein	0.9
302	Brown Swiss	3.0
405	Holstein	1.3

TABLE 3

*Influence of preservatives on the lactic acid value of fresh milk and their effectiveness of preservation*

Preservative	Concentration of preservative	Lactic acid value	
		Before storage	After storage
	<i>per cent</i>	<i>mg./100 gm.</i>	<i>mg./100 gm.</i>
Control .....	0.00	1.9	19.8
Mercuric chloride* .....	0.24	2.2	1.7
Formalin .....	0.10	1.7	1.9
Control .....	0.00	3.5	31.7
Potassium dichromate .....	0.50	2.1	1.7
Boric acid .....	0.75	3.5	3.2
Control .....	0.00	2.2	15.8
Sodium benzoate .....	0.75	21.1	26.8
Phenol .....	0.20	2.1	4.6

\* Commercial corrosive sublimate—true mercuric chloride concentration considerably less.

of fresh milk is essentially unaffected by the preservatives tested. The concentration of dichromate was considerably higher than is generally used for preservation of milk, which may account for the lower result for lactic acid obtained when this preservative was used. Sodium benzoate apparently increased greatly the lactic acid value of the milk and therefore appears to be an unsatisfactory preservative for samples which are to be analyzed by this technique.

These data also indicate that, with the concentrations used, mercuric chloride, formaldehyde, potassium dichromate, and boric acid prevented lactic acid development during storage of the milk, whereas sodium benzoate and phenol were less effective. In one series, the mercuric chloride and formaldehyde-containing samples (following their storage at 37° C. for several hours) were held at 5° C. for 12 days. The lactic acid content did not increase during this period of time.

TABLE 4

*Influence of lipolysis on the lactic acid content of milk*

Sample	Lactic acid value	
	Before storage	After storage
	<i>mg./100 gm.</i>	<i>mg./100 gm.</i>
Series I		
Sample 1 .....	2.3	2.5
Sample 2 .....	1.7	2.6
Sample 3 .....	2.0	2.3
Series II		
Sample 1 .....	3.3	3.2
Sample 2 .....	3.2	3.0
Sample 3 .....	2.7	3.0



*Influence of lipolysis.* The effect of the development of rancidity on the lactic acid determination was determined by resorting to two somewhat different procedures. In one procedure (Series I), three different samples of raw milk were mixed in equal proportion with pasteurized, homogenized milk. This procedure was shown previously to produce rancidity within a few hours (6). Formalin was added at the rate of 0.1 per cent to prevent bacterial activity. Lactic acid measurements were made immediately after mixing the non-homogenized and homogenized portions and again after the mixtures had been held at 4° C. for approximately 6 hours.

In another procedure (Series II) three samples of raw milk were homogenized at 2500 pounds pressure and stored for 48 hours at 4° C. Formalin was added as in Series I to prevent bacterial spoilage. The lactic acid content of the milk was measured at the beginning and after the 48-hour storage.

The results of the lipolysis studies are presented in table 4.

TABLE 5

*Influence of ethyl alcohol in the extracting ether or in the milk on lactic acid recovery\**

Concentration of alcohol		Lactic acid value of milk when alcohol added to	
In ether	In milk	Ether	Milk
<i>per cent</i>	<i>per cent</i>	<i>mg./100 gm.</i>	<i>mg./100 gm.</i>
0	0	41.4	10.3
3	1	44.8	10.1
6	2	44.8	10.0

\* Lactic acid was added to milk at rate of 39 mg./100 gm. in the ether trials and at rate of 9 mg./100 gm. in the milk trials.

In general, the data in table 4 reveal no appreciable influence of fat hydrolysis on the lactic acid determination. In other experiments, as much as 2 per cent glycerol was added to milk without altering the lactic acid results.

*Influence of ethyl alcohol in ether and in milk.* Since observations had revealed that the use of impure ether (U.S.P. grade) for extraction resulted in appreciably reduced recovery of added lactic acid, trials were conducted to determine if the presence of alcohol in the ether was the vitiating factor. Also, a study was made of the recovery of lactic acid from milk to which both lactic acid and ethyl alcohol had been added.

The results are presented in table 5.

The results show that the addition of either 3 or 6 per cent alcohol to the ether used for extraction resulted in an increase of approximately 8.2 per cent in the lactic acid recovered. This would indicate that the low recovery of lactic acid when impure ether is used is not due to the presence of ethyl alcohol in the ether.

TABLE 6

*Influence of the addition of salts or sucrose upon the lactic acid content of milk*

Sample	Amount added	Lactic acid content
	<i>per cent</i>	<i>mg./100 gm.</i>
Control .....	0.00	2.5
Sodium chloride .....	0.25	2.5
Sodium chloride .....	0.50	2.9
Control .....	0.00	2.4
Sodium citrate .....	0.20	2.2
Disodium phosphate .....	0.20	2.4
Control .....	0.00	2.6
Sucrose .....	5.00	2.3
Sucrose .....	10.00	2.9
Control* .....	0.00	22.6
Sucrose* .....	5.00	22.8
Sucrose* .....	10.00	22.4

\* Lactic acid added to milk samples.

The results in table 5 also reveal that the addition of ethyl alcohol to milk in concentrations of 1 or 2 per cent did not appreciably affect the lactic acid recovery.

*Influence of salts and sugar.* Trials were conducted in which sodium chloride, sodium citrate, disodium phosphate and sucrose were added to milk. The lactic acid values for these trials are presented in table 6. The results reveal that these compounds, in the concentrations used, have no influence on the lactic acid values as determined by the Hillig procedure.

*Influence of neutralization.* Milk was permitted to undergo appreciable souring and was then divided into two lots. One lot was neutralized either with sodium carbonate or sodium bicarbonate to approximately the original pH and the second was neutralized to somewhat over pH 7. The results are presented in table 7.

These data indicate that neutralization of milk with sodium bicarbonate or sodium carbonate slightly increases the lactic acid values as measured

TABLE 7

*Influence of neutralization on the lactic acid values of milk*

Sample	pH	Lactic acid values
		<i>mg./100 gm.</i>
Neutralizer— $\text{NaHCO}_3$		
Fresh milk .....	6.58	.....
Soured milk .....	6.32	57.9
Neutralized .....	6.68	62.6
Overneutralized .....	7.25	65.8
Neutralizer— $\text{Na}_2\text{CO}_3$		
Fresh milk .....	6.56	.....
Soured milk .....	6.29	53.2
Neutralized .....	6.59	55.2
Overneutralized .....	7.27	58.4

by the Hillig technique. Similar results have been obtained when neutralization was with disodium phosphate.

#### DISCUSSION

Results secured in this study indicate that the Hillig colorimetric method for determining lactic acid in milk is reliable and accurate when applied to milk containing up to approximately 100 mg. per cent of lactic acid, thus agreeing closely with the results presented by Hillig (1). On this basis the method appears to be a definite contribution to the determination of quality in dairy products.

Application of this test (or a test of similar accuracy) to quality control by state and municipal regulatory officials would be highly desirable. However, the time and skill required for making the determination may limit its general application. Approximately eight hours are necessary for making an analysis, about one-half of this being utilized for ether extraction of the serum. The time disadvantage may be overcome somewhat by having a series of extractors operating simultaneously.

One of the major advantages of the colorimetric method is its constancy. Several factors which have been reported by Troy and Sharp (7) to influence the aldehyde method were without effect upon the colorimetric method. Thus, the method may be utilized in analyzing (a) products high in sugar, (b) products which have undergone considerable lipolysis, and (c) milk products which have been preserved for long periods with mercuric chloride. The accuracy of the method for preserved samples is of especial value to a laboratory relying upon preservation of field samples.

Another desirable feature of the colorimetric method is the elimination of a titration procedure. This is of advantage in a laboratory doing routine analyses where, once the procedure is standardized, considerable technical skill is not required to conduct the test. However, this presupposes the use of a photoelectric colorimeter for measuring color intensity. In fact, the use of a photometer rather than an ordinary visual colorimeter or Nessler tubes would appear to be essential if a high degree of accuracy is to be obtained with low concentrations of lactic acid, since a visual matching of the yellow color produced is not easy.

#### SUMMARY AND CONCLUSIONS

The Hillig colorimetric lactic acid determination usually gave recovery of lactic acid from milk within 0.001 per cent. Freshly drawn milk was found to give values equivalent to 0.9 to 3.0 mg. lactic acid per 100 grams milk by this method. These values probably represent the blank for strictly fresh milk rather than lactic acid.

The colorimetric method was unaffected by the concentrations of mercuric chloride, formalin, boric acid and phenol employed in this study; by

development of rancidity; or by the addition to the milk of certain concentrations of sodium chloride, sodium citrate, disodium phosphate, sucrose, and ethyl alcohol. Sodium benzoate markedly increased the lactic acid value and potassium dichromate appeared to have a slight depressing effect.

Neutralization appears to slightly increase the lactic acid values obtained by this method.

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## LACTIC ACID IN DAIRY PRODUCTS. II. RELATION TO FLAVOR, ACIDITY MEASUREMENTS, BACTERIAL COUNT AND METHYLENE BLUE REDUCTION\*

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The lactic acid content of concentrated milk products reflects the quality of the raw milk used in their manufacture, and application of lactic acid determinations is now being made with this in mind. The present problem of the manufacturers of evaporated, condensed and dried milk is to grade the raw milk supply so that the final product will not contain an excessive amount of lactic acid. To accomplish this, use of lactic acid measurements on the raw milk would be the ideal procedure, but this is impractical because of the time and skill required for the determination. However, the plant operator may establish standards with more practical grading methods that will insure low lactic acid values in the manufactured products.

Practical methods commonly used for the grading of the raw milk supply include (a) the direct microscopic test, (b) the methylene blue reduction test, (c) the organoleptic test, *i.e.*, odor and taste, and (d) titrable acidity. Much information is available relative to the use of these procedures to determine quality in milk, but their applicability to a grading program having a lactic acid basis has been incompletely ascertained. It was for the purpose of securing more information along this line that the investigation reported in this paper was conducted.

Various studies have been conducted to determine the relationship between acidity measurements and acid flavor. As early as 1891, Stokes (14) found milk containing various preservatives to taste sour when the titrable acidity was from 0.4 to 0.49 per cent, whereas unpreserved milk usually tasted sour when the acidity reached 0.3 per cent. Richmond and Harrison (8) in 1900 observed milk to taste sour at an acidity of 0.405 per cent. Later (1918) Van Slyke and Baker (16) made observations at frequent intervals of milk undergoing souring and, when the first perceptible characteristic sour milk flavor was detected, determined the pH and titrable acidity. The results on seven samples indicated that appreciable acidity had developed in the majority of the samples by the time the flavor was detected, with pH values ranging from 5.44 to 5.96 (decidedly below the normal pH range of 6.3–6.7) and with the titrable acidity ranging from approximately 0.33 to 0.13 per cent, expressed as lactic acid. Four of these samples were permitted to sour naturally at 25° C. At the first signs of souring the pH values ranged from 5.49 to 5.44, and the titrable acidity from approxi-

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mately 0.22 to 0.33 per cent. These workers concluded that there was no relationship between the development of the characteristic sour-milk flavor and the pH and titrable acidity.

Hammer and Hix (5) inoculated aseptic milk with strains of *S. lactis* and determined the acidity changes and bacterial plate counts in milk when a change in flavor appeared. These workers differentiated between the "sour" flavor and the flavor distinguished "by acidity increases only" and could find no definite relationship between the development of "sour" taste and increase in acidity. In certain cases, milk showing acidity increases of 0.04–0.05 per cent was classed as "sour" by taste whereas other milk with the same acidity changes was classed as showing only a distinct rise in acidity. One sample was considered to be "sour" when the acidity had increased 0.03 per cent, and another with an acidity increase of 0.02 per cent was graded as having a distinct rise in acidity. Their results reveal, however, that when the milk exhibited either a rise in acidity by taste or a "sour" flavor, the actual titrable acidity had increased in every case, the increases varying from 0.02 to 0.08 per cent and with the majority varying from 0.04 to 0.06 per cent, expressed as lactic acid.

These workers found only a general relationship to exist between acidity increases and numbers of organisms present, but noted that large increases in bacteria had occurred by the time acidity changes were detected by taste. In one series, seven samples which exhibited definite rise in acidity gave plate counts ranging from 30 to 90 million bacteria per ml., and four "sour" samples (by taste) possessed counts of 53 to 116.5 million bacteria per ml. In another series, those samples showing a rise in acidity had bacterial counts of 37.5 to 127 million per ml., and those which were classed as sour showed bacterial counts of 91 to 235 million per ml.

Schultz, Marx and Beaver (9) determined the pH of milk colorimetrically and correlated the findings with bacterial count. The conclusions were that the bacterial count of milk begins to increase markedly at pH 6.6 (approximately 16 million per ml.) and at pH 6.5 the count reached an average of 100 million per ml. The acidity was first detected by taste at about pH 6.0. Analysis of some of the data of Cooledge and Wyant (1) reveals the milk to have a "sour" flavor at pH values ranging from 5.8 to 6.4, although the authors made no attempt to correlate the pH with the first changes in milk flavor.

Sommer and Hart (11) indicate that an increase of 0.03 per cent titrable acidity in milk represents the maximum increase permitted in grading milk, and Sommer (10) observes that acidity increases of 0.02 per cent and sometimes as high as 0.05 per cent are necessary before a "sour" odor is detectable.

Although it is often the practice to class milk flavors associated with acid development under the heading of "high-acid" or "sour," considerable

doubt has been expressed that the flavor produced is necessarily related to lactic acid formation. Van Slyke and Baker (16) refer to the first perceptible sign of souring as "a characteristic flavor, discernible to the senses of both smell and taste, due to the presence of some volatile compound formed in the souring process and not to lactic acid. The flavor appears before the milk begins to taste acid." They further indicate that the flavor is the result of bacterial action, and the compounds responsible unidentified.

Sommer (10) also points out that the "sour" odor is not due to lactic acid but to volatile substances such as acetic, propionic and formic acids, acetaldehyde, acetone, diacetyl and methylacetylcarbinol, and that the proportion of these to lactic acid governs the extent to which milk must sour before the change is detected by odor.

Another type of flavor which is closely associated with acid development is the "malty" flavor. Some milk judges class this flavor defect as a high-acid or sour-milk flavor, whereas others refer to it as a "bacterial" flavor. The flavor has been found to be produced by a streptococcus (4), by a micrococcus alone and in cooperation with a bacillus (15), and may also result through the action of lactobacilli (13).

Hammer and Cordes (4) found a streptococcus (*S. lactis* var. *multigenes*) to produce the malty or burnt flavor, and studied the development of the flavor in sterile milk by inoculation with the causative organism. Titrable acidity values on the milk when the flavor was first observed ranged from 0.25 to 0.38 per cent. They stated that "it is evident that the caramel (or malty) flavor developed at a low acidity, although the flavor was never noticed until there had been a definite increase in acid." Hammer (3) later stated that, "the organisms can produce a malty condition with no significant increase in acidity, and sweet milk or cream is sometimes distinctly malty, although sour products are more commonly involved."

The maltiness produced by micrococcus was found by Tracy and Ramsey (15) to be intensified when a spore-forming rod was present. The flavor was found in milk in which the titrable acidity was usually not above 0.18 per cent. In two trials with methylene blue, malt-flavored milk with a bacterial count of 700,000 per ml. reduced the dye in 2.25 hours in one case, whereas in another case, when the count was 1.6 million per ml., the reduction time was 1.75 hours. The most typical flavor was produced at 68° F., but higher temperatures of 80° F. and 100° F. developed the flavor more rapidly. Total bacterial counts of samples incubated at 68° F., 85° F. and 100° F. were approximately 5.7 million, 10.4 million and 9.8 million per ml., respectively, when the malt-like flavor was detected.

#### GENERAL EXPERIMENTAL PROCEDURE

The milk used in this study was secured from the raw milk supply of the Michigan State College creamery and represents a wide variety of sources.

The milk was warmed to the specified temperature (either 32–35° C. or 22–24° C.) and observations made at frequent intervals for flavor changes.

The organoleptic examination of the milk was accomplished by at least two judges working independently. The milk was judged on the basis of the appearance of any acid-associated flavors. Thus, the classification included such flavors as "malty" as well as the typical acid or sour-milk flavors. Agreement between the judges was necessary before any sample was deemed to have an acid-associated flavor.

In addition to taste-odor determinations, titrable acidity and lactic acid measurements, the direct microscopic clump count and the methylene blue reduction test were made.

Lactic acid determinations were conducted essentially by the Hillig technique (6, 7) with certain modifications (2). When it was impractical to analyze for lactic acid immediately, the sample was preserved with mercuric chloride (added at a concentration of 0.3 per cent) and stored at 4° C. The samples were held usually no longer than 48 hours. Results of the lactic acid determinations are referred to as "lactic acid values" unless correction is made for the residual or blank value equivalent to 1–3 mg. lactic acid/100 gm., characteristic of strictly fresh milk (2).

Titration acidity was ascertained by titrating an 18-gm. sample with 0.05 N NaOH solution to the phenolphthalein end-point, a method considerably more sensitive than the ordinary procedure which involves a 9 gm. sample and 0.1 N NaOH. In addition, titrations were conducted against a white background with good lighting afforded by a desk lamp with a day-light bulb, which was adjusted directly over the samples.

The methylene blue reduction test and the microscopic bacterial clump count were conducted in accordance with Standard Methods (12). All samples were placed in test tubes and held in ice water for 20–30 minutes just prior to adding the dye or before shaking for making the smear for the direct microscopic count. The microscopic clump count was made independently by both authors.

#### EXPERIMENTAL RESULTS

##### (a) *Effect of Storage at 32–35° C.*

*Flavor and lactic acid.* In the first trials which were conducted, raw milk from various sources was held at 32–35° C. Samples of the milk were taken at the start of the incubation period and at intervals thereafter until the milk had developed pronounced acid-like flavors. Results showing relationships between the flavor and lactic acid content of the milk are presented in tables 1 and 2.

The total lactic acid content of the milk samples is shown in table 1. These results reveal the relatively low content of lactic acid required before the acid-like flavors appeared. In general, under these conditions, the criti-



TABLE 1

*Correlation of acid-associated flavors with the lactic acid values when the milk was incubated at 32-35° C.\**

Flavor of samples	Number of samples having lactic acid values of					
	0-1.9	2-2.9	3-4.9	5-9.9	10-14.9	15 or more
Normal .....	6	16	9	0	1	0
Acid .....	1	2	8	15	5	7

\* Lactic acid expressed in milligrams per 100 gms. milk.

cal lactic acid content was approximately 5 mg./100 gm. since at values above this concentration all of the samples (with one exception) were classed as having acid-like flavors. However, these data show that samples having such flavors may have lactic acid values below that which is normal for strictly fresh milk. For example, three samples which contained less than 3 mg./100 gm. milk of lactic acid were criticized for having acid-like flavors. In another series conducted under similar conditions, four out of twelve samples were found to have acid flavors at these low lactic acid concentrations.

As a rule, there was a fairly good relationship between the intensity of the developed flavor and the lactic acid content. At lactic acid values above 10 mg./100 gm., the judges usually classed the flavor as "pronounced" or "strong," whereas at lower values, "slight" and "distinct" were the usual designations.

In table 2 is shown the relationship between lactic acid production and acid-type flavors. Lactic acid production represents the difference between the original and final lactic acid content of the milk. The results of these 44 samples reveal lactic acid to have been produced in every case by the time the sample was classed as having an acid-associated flavor, but that the amount of acid involved was slight. All but one of the samples which had developed 5 mg. or more of lactic acid possessed acid-like flavors, and 36, or approximately 82 per cent, of all samples which showed lactic acid increases, were classed as acid. Of the 30 samples which developed more than 2 mg.

TABLE 2

*Correlation of acid-associated flavors with production of lactic acid when the milk was incubated at 32-35° C.\**

Flavor of samples	Number of samples having lactic acid increases of					
	0-0.9	1-1.9	2-2.9	3-4.9	5-9.9	10 or more
Normal .....	5	1	0	1	1	0
Acid .....	2	6	6	2	14	6

\* Lactic acid expressed in milligrams per 100 gm. milk.

of lactic acid per 100 gms., 28 were designated as having acid-associated flavors.

The question may be raised whether these samples had already undergone some lactic acid formation before the trials were started. This would appear to be a logical question, since the milk samples were selected at random from milk as it was delivered from various farms to the plant. However, that appreciable lactic acid production had previously occurred is highly unlikely, because the initial lactic acid values of the samples were within the normal range obtained for fresh milk by this method with an average of 2.5 mg./100 gm.; also, the bacterial counts were too low to indicate bacterial fermentation.

It was observed that the "malty" or "bacterial" type of flavor was common. This may be expected in view of the findings of Hammer and Cordes (4) that warm temperatures favor the production of maltiness by strepto-

TABLE 3

*Correlation of bacterial clump count with lactic acid production in milk held at 32-35° C.*

Lactic acid increase mg./100 gm.	Number of samples having microscopic clump counts* of					
	Less than 2	2-2.9	3-4.9	5-9.9	10-19.9	More than 20
0- 2.0	7	4	3	1	1	...
2- 3.9	1	4	...	1	1	...
4- 5.9	...	1	1	1	3	1
6- 7.9	...	1	...	1	...	2
8- 9.9	...	...	1	...	...	...
10-14.9	...	...	1	1	...	...
15-19.9	...	...	...	1	...	...

\* Bacterial count in millions.

cocci. However, the predominant organism in the majority of the "malty" samples appeared to be a bacillus.

*Bacteria, lactic acid and flavor.* Correlation of the bacterial clump count with the lactic acid production is presented in table 3 and reveals only a general relationship. The relationship would be affected by differences in lactic acid fermenting capacity of the organisms involved and also by the fact that these organisms vary greatly in their clumping ability, thus directly influencing the microscopic count. These results do indicate, however, that appreciable bacterial multiplication had occurred by the time two or more milligrams of lactic acid had been formed, since the milk samples usually had initial bacterial counts well under 200,000 per ml. and often had counts of less than 50,000 per ml.

The relationship between the methylene blue reduction time and lactic acid formation is shown by the data in table 4.

These results reveal that when four milligrams or more of lactic acid had been formed, the methylene blue reduction time was always less than one

TABLE 4

*Correlation of lactic acid production with methylene blue reduction time when the milk was held at 32-35° C.*

Lactic acid increase  mg./100 gm.	Number of samples having methylene blue reduction time of			
	More than 3.5 hrs.	1-3 hrs.	30 min.-1 hr.	Less than 30 min.
Less than 2 .....	2	8	4	...
2-3.9 .....	...	3	1	2
4-9.9 .....	...	...	8	5
10 or more .....	...	...	...	6

hour, and usually was less than 30 minutes. Although the relationship between bacterial count and methylene blue reduction time is not shown, it was observed that all samples having more than two million bacteria reduced the dye within three hours, and usually in less than one hour. The methylene blue results, however, represent somewhat abnormal conditions since the milk was held at 32-35° C., and had been placed in ice water only 15-30 minutes prior to starting the test. Thus, the organisms were in a highly active state, and would be expected to reduce the dye rapidly.

Another comparison made in these trials involved the microscopic clump count and acid-associated flavors. These results are shown in table 5.

These data reveal that the clump count of milk is relatively low when acid-associated flavors are first perceptible. The majority of samples having counts above two million were graded as acid-flavored; in fact, six samples which possessed acid-like flavors had bacterial counts of less than two million. All samples possessing a clump count of more than five million had acid-associated flavors.

*(b) Effect of Storage at 22-24° C.*

Since the possibility exists that storage at 32-35° C. may produce changes which are more or less specific for these temperatures, it appeared desirable to conduct somewhat similar trials in which the milk was held at lower temperatures.

TABLE 5

*Correlation of bacterial clump count with production of acid-associated flavors when milk was held at 32-35° C.*

Flavor of samples	Number of samples having microscopic clump counts* of						
	Less than 0.5	0.5-9	1-1.9	2-2.9	3-4.9	5-9.9	10 or more
Normal ...	18	7	2	0	1	0	0
Acid .....	0	0	6	13	5	8	11

\* Bacterial counts expressed in millions.

In these trials, samples of milk were taken at random as received at the College Creamery, examined for flavor, and analyzed for titrable acidity. They were then stored overnight at 4° C. Early the following morning, the samples were warmed to 22–24° C. and examined at intervals for the pres-

TABLE 6

*Relationship between the total lactic acid content of milk and the production of malty and high-acid flavors, the bacterial clump count and the methylene blue reduction test when the milk was held at 22–24° C.*

Sample No.	Acid-associated flavor*	Lactic acid content	Microscopic clump count		Methylene blue reduction time
			Number	Predominant type†	
		mg./100 gm.	millions -		hours
1	Malty +	2.5	1.4	Rods	2.8
2	" ++	1.6	3.3	Rods, cocci	1.5
3	" ++	4.5	6.4	Rods	2.3
4	" ++	2.2	2.8	Rods, cocci	3.5
5	" +	2.3	3.0	Rods, cocci	2.5
6	" +	2.5	5.2	Rods	1.7
7	" +	4.6	6.9	Rods, cocci	0.9
8	" +	4.6	2.9	Rods, cocci	2.5
9	" ++	2.9	6.1	Rods, cocci	1.5
10	" ++	4.6	6.7	Rods	0.9
11	" ++	5.7	6.6	Rods, cocci	0.9
12	" ++	3.8	4.5	Strep.	2.5
13	Malty, high-acid +	2.3	7.5	Rods, cocci	0.7
14	" " +	2.9	2.4	Rods, cocci	3.3
16	" " +	4.8	1.5	Rods, cocci	2.5
17	" " ++	.....	12.0	Rods, cocci	1.0
18	" " +	.....	3.8	Rods, cocci	1.5
19	" " +	2.5	1.3	Rods, cocci	2.0
20	" " +	.....	2.3	Strep., staph.	1.9
21	" " +	8.2	7.3	Rods, cocci	0.9
22	" " ++	3.1	14.3	Rods, cocci	0.9
23	" " +	4.1	4.5	Rods, cocci	1.5
24	" " ++	4.6	19.1	Rods, cocci	0.7
25	" " +	3.1	6.6	Rods	0.9
26	High-acid ++	10.9	20.0	Strep.	0.3
27	" +	3.6	10.0	Strep., staph.	1.2
28	" +	10.2	18.8	Strep., rods	2.3
29	" +++	25.0	61.4	Strep.	0.3
30	" +	5.6	21.0	Strep.	0.7
31	" +++	16.9	62.0	Strep.	0.3
32	" +	4.7	11.7	Strep., rods	0.9
33	" +++	25.9	160.0	Strep.	0.5
34	" +++	24.4	131.0	Strep.	0.3
35	" ++	18.2	68.5	Strep., rods	1.0

\* Intensity of flavor indicated by number of plus signs: + slight; ++ distinct; +++ pronounced.

† Unless otherwise indicated, "cocci" refers to various types of coccus-formed bacteria other than streptococci, i.e., micrococci, diplococci, and in certain cases, staphylococci.

ence of acid-associated flavors. When a sample exhibited such a flavor it was promptly subjected to the various quality tests.

*Lactic acid, flavor, bacterial tests.* The examination of these samples for developed flavors revealed that although the malty flavor appeared fre-

quently, as in the case of the higher storage temperature, there were more samples in which a typical acid flavor developed. This high-acid flavor appeared to be more difficult to detect than those flavors of the malty type and, therefore, was associated with higher titrable acidity increases and with much greater bacterial counts. In these trials, the judges attempted to differentiate between the flavors by the terms "malty" and "high-acid." The results showing the relationship between the total lactic acid content of milk and the flavors, bacterial count, and methylene blue test, are presented in table 6.

In this table, the samples were grouped in accordance with the flavor defect indicated by the judges, *i.e.*, malty, malty-high-acid and high-acid. However, recognition should be given to the fact that it is not always possible to differentiate between these various acid-associated flavors. The data in table 6 indicate that for the same intensity of off-flavor, the high-acid samples had undergone more lactic acid formation and possessed definitely higher bacterial counts than those samples having the malty-type flavor. There are, however, some exceptions.

There is no appreciable difference between the lactic acid values and bacterial counts of the two groups classed as "malty" and "malty-high-acid." Of the 12 samples possessing slight or distinct malty flavors, the lactic acid content ranged from 1.6 mg. to 5.7 mg./100 gm. and the bacterial counts ranged from 1.4 million to 6.9 million/ml. In the "malty-high-acid" group, the lactic acid content ranged from 2.3 mg. to 8.2 mg./100 gm. and the bacterial count ranged from 1.3 million to 10.1 million/ml. The average values for all of these samples having at least some semblance of a malty flavor were as follows: Lactic acid content, 3.7 mg./100 gm.; bacterial count, 5.7 million/ml.; methylene blue reduction time, 1.7 hours.

Although the milk was examined at frequent intervals, several of the "high-acid" samples had developed a pronounced flavor by the time of detection, probably because of the extremely high bacterial counts and the accelerated bacterial activity. The flavor production likely occurred rapidly between observation periods. It is interesting to note that in those samples having pronounced high-acid flavors, the bacterial count ranged from 61.4 million to 160 million/ml. and the lactic acid content ranged from 16.95 mg. to 25.90 mg./100 gm. Average values for the 10 samples characterized as "high acid" were as follows: Lactic acid content, 14.5 mg./100 gm.; bacterial count, 56.4 million/ml.; methylene blue reduction time, 0.8 hour.

Another interesting deduction which may be drawn from the results in table 6, pertains to the type of organism which predominates in production of the flavors. In those samples having malty-like flavors, rod-shaped organisms were involved in all but one case, and they were usually accompanied by smaller numbers of cocci of one or more types. It should be emphasized, however, that the organism which predominates under conditions such as

exist in this experiment may be influenced by the temperature and time of incubation.

The correlation between bacterial count and lactic acid production is seen to be only fairly general. For example, in one case, a sample with a bacterial count of 131 million/ml. had a lactic acid value of 24.4 mg./100 gm. in contrast to another sample with a count of 61 million/ml. and a lactic acid value of 25 mg./100 gm. In both of these samples, however, the bacterial count and the lactic acid content were comparatively high. If these were compared with a sample having a bacterial count of 10 million, a definite difference in the lactic acid values would be expected.

The results with the methylene blue test indicate that, under the conditions of this experiment, milk having a high lactic acid content will usually exhibit short reduction periods. For example, milk with lactic acid value of 15 mg./100 gm. or more, reduced the dye in one hour or less. However, milk having far less lactic acid may also reduce the dye quickly. It does appear

TABLE 7  
*Increases in lactic acid as measured by titrable acidity and by lactic acid methods\**

Range in lactic acid content	No. of samples	Average lactic acid increase		Range of individual sample variation (b) from (a)
		By lactic acid method (a)	By titrable acidity (b)	
<i>mg./100 gm.</i>		<i>mg./100 gm.</i>	<i>mg./100 gm.</i>	<i>mg./100 gm.</i>
4.9 or less .....	36	1.6	2.4	0.3-10.3
5.0- 9.9 .....	18	6.8	8.5	0.2-13.3
10.0-14.9 .....	3	14.3	13.5	0.6- 9.7
15.0-19.9 .....	4	17.9	22.2	0.2-14.8
20 or more .....	5	22.8	26.7	1.4- 6.5

\* Titrable acidity determination was conducted on an 18-gm. sample with 0.05 N NaOH.

likely, however, that milk which reduces the dye within 30 minutes will contain appreciable quantities of lactic acid.

*Titrable acidity and lactic acid.* At the beginning of this investigation, an effort was made to use both pH measurements and the titrable acidity test to measure acid production in the milk. However, the pH determinations were soon discontinued when they were found to be no more sensitive than the titration test in detecting slight acidity changes.

In the case of acidity measurements, it is fully realized that these determinations are of no value insofar as establishing grading standards therefrom is concerned, since normal variations in titrable acidity far exceed the changes required to produce appreciable quality deterioration in milk. However, the principal purpose of such determinations was to permit a direct comparison between acidity increases as measured by titration and as measured by the more sensitive lactic acid technique.

As previously indicated, the titrable acidity determination involved an 18-gm. sample and 0.05 N NaOH. The results of 66 trials in which compari-

sons were made between increases in lactic acid and increases in the titrable acidity (also expressed as lactic acid) are summarized in table 7. In this table, data are arranged to indicate the variation of the titrable acidity from the true lactic acid content at different levels of lactic acid increases.

These data reveal a fairly close relationship to exist between the two methods when the average results are considered, with a maximum difference of approximately 4 mg./100 gm. However, the data also show that in the case of individual samples, the titrable acidity results may vary rather widely from the true lactic acid content, the maximum variation amounting to 14.8 mg. lactic acid per 100 gm. milk.

Although the results of the titration and lactic acid methods on individual milks are not shown, the former was found to give lower results in 29 of the 66 samples, or in 43.9 per cent of the total determinations. Furthermore, it was observed that the extreme variations between the two methods occurred in those cases in which the titration values were higher. In samples in which the titration results were lower than obtained by the lactic acid procedure, the maximum variation was approximately 5 mg. lactic acid/100 gm. However, in the 37 samples in which the titration indicated a higher lactic acid content than shown by the lactic acid method, the maximum difference between the methods was 14.8 mg./100 gm., and seven samples varied by more than 8 mg./100 gm.

Two factors are likely involved in creating the variations between these two methods on individual samples: (a) the lack of accuracy on the part of the titration procedure, and (b) the possibility that the development of rancidity in certain samples before the acid flavor appeared may have influenced the titration result. Even by using the titration technique involving larger samples, weaker alkali solution, and excellent lighting, the titration method is still subject to some end-point errors and variations. That the titration results agree to the extent indicated in table 7 is surprising.

From the rancidity standpoint, certain samples did possess rancid flavors early in the storage period, and the development of rancidity may be expected to increase the titrable acidity without affecting results by the lactic acid determination. This factor is likely responsible for the major differences between the two methods.

#### DISCUSSION

When consideration is given to the grading of the raw milk supply on the basis of the lactic acid content, the question which arises is, "When is milk sour?" and the answer to the question will likely be an important consideration if and when quality standards involving lactic acid are established. However, at the present time a direct simple answer to this question is difficult, if not impossible, because any such answer must be qualified in one manner or another as dictated by the various factors involved.

One factor to be considered in this connection concerns the methods used for determining the degree of "sourness" or "sweetness" of milk. Under present conditions, milk that is deemed to be sour by one method may not be classed as sour by another. Therefore, if the extent of sourness is to be used as the basis of a grading program in the concentrated milk field, then the various practical quality methods now in use should be reinvestigated with the view of establishing standards for each of them on this basis.

Organoleptic detection of sourness in milk has been the subject of much discussion, and its reliability is questioned because of (a) differences in the acuteness of the individual's senses of taste and smell; (b) the presence of other flavors which may interfere with sour flavor detection; (c) peculiarities of specific flavors produced by different microorganisms, these flavors being detectable at varying levels of acid development. Notwithstanding, the results obtained in this study indicate that, when properly applied, the organoleptic method is highly sensitive and detected acid-type flavors which occurred as a rule at much lower lactic acid levels than have been reported previously. Generally, when acid-associated flavors were perceptible, lactic acid increases were below 10 mg./100 gm. and often were not over 5 mg./100 gm. In fact, lactic acid increases of 2 mg./100 gm. were not uncommon in milk having acid-associated flavors.

On the basis of available information, it appears that a grading standard which permits an increase in the milk of 0.03 per cent acidity (30 mg./100 gm. of lactic acid) is indeed generous if the grading is to be conducted by well-trained milk graders. This amount of formed acid is considerably above the critical concentration for flavor production. It is appreciated, however, that a certain tolerance above the critical lactic acid content found in this study is desirable, since it is not always feasible to have the grading conducted by highly skilled milk judges. In addition, more information is desirable on the flavor-acid relationship as influenced by different microorganisms before establishing stringent lactic acid standards.

As has been pointed out by previous workers, the actual taste and odor detectable at the first signs of souring are probably due to compounds other than lactic acid. However, the results herein reported indicate that lactic acid has been formed in practically all, if not all, samples of milk which possess an acid-associated flavor, although the amount formed is often slight.

The type of flavor produced in milk during souring is a factor to be considered in arriving at conclusions regarding organoleptic grading of such milk. The authors have often observed that flavors of the malty type are detected at lower lactic acid content and lower bacterial count than those flavors which are more strictly of the high-acid type, doubtless due to the larger volume of aroma associated with the malt-flavor production. This field of study needs further exploration before final conclusions may be drawn.



One point of contention in milk grading relates to the terminology used in designating the sour-milk flavors. The term "high-acid" is objected to by many because the milk is within the normal range of titrable acidity. Furthermore, the term "high-acid" indicates an acid-like flavor, whereas there is no semblance of an acid flavor in the earlier stages of souring. It would appear logical, at least for a quality program, to classify all of those flavors which occur when milk undergoes souring as one general type, and avoid the confusion which may result by differentiation. The principal point involved is that these flavors are all produced by bacterial activity coincident with the formation of lactic acid and the appearance of any of these flavors indicates milk of inferior quality. The term "acid-associated" would appear to be suitable for all of those flavors which result from or occur simultaneously with lactic acid formation.

The relationship found in this investigation between bacterial count and flavor appears to be somewhat different than has been indicated previously. The results do reveal that acid-associated flavors are commonly present in milk having bacterial clump counts of 2-5 million per ml. These comparatively low values, considerably under the plate counts reported by Hammer and Hix (5), may be the result of (a) detecting the flavor changes at lower acid increases, (b) allowing the milk to develop the flavor normally with a wide variety of organisms being present and without resorting to artificial inoculation, and (c) differences in the types of organisms involved. The results herein presented indicate that the production of about 0.02 per cent titrable acidity (20 mg. lactic acid/100 gms. milk) by a predominance of streptococci, gave data similar to those of Hammer and Hix (5).

Variation in bacterial counts may be normally expected and, in this study, milk samples exhibited widely different microscopic clump counts at the first detectable flavor change. These variations, in general, are governed by the type of organism predominating in the respective samples and also by the temperature of incubation. Certain organisms possess considerable clumping properties and remain in clumps during the preparation of the sample for staining. Other organisms, particularly the streptococci found in these samples showing a typical "high-acid" flavor, were more widely distributed over the microscopic field. Thus, in two samples, the individual cell count may be similar, but due to the clumping properties of the predominant organism in one sample, the clump counts may be markedly different.

The titrable acidity test as it is now used will probably never be of any great value as an accurate control test, since the amount of the titer which is contributed by lactic acid is not easily ascertained. In fact, the common titration procedure which involves the use of a 9-gm. sample and 0.1 N NaOH solution would appear to be of little value even as an adjunct to organoleptic examination unless the sample in question had undergone

acidity changes far in excess of those required for production of acid-associated flavors. For example, the results of these experiments indicate that the milk usually was of inferior quality as determined by any of the quality tests when the lactic acid increase amounted to 0.010 per cent or less. Since the titrable acidity of mixed herd milk may normally vary by several times this quantity, it may readily be appreciated that the establishment of grading standards based on titrable acidity measurements is unfeasible.

In connection with acidity values, the results herein presented reveal a general relationship between acid increases as measured by a sensitive titration method and by the lactic acid method. However, under normal conditions, even a sensitive titration method should serve only as an indicator of acidity changes rather than as a precise instrument for measuring lactic acid changes, since titrable acidity increases on individual milk samples may vary appreciably from the true lactic acid content.

#### SUMMARY AND CONCLUSIONS

Results are presented of the relationship of the formation of lactic acid in milk with (a) the production of acid-associated flavors, (b) the microscopic clump count, (c) the methylene blue reduction, and (d) titrable acidity.

Acid-associated flavors were detected at low levels of lactic acid formation. Many samples of milk exhibited such flavor defects at lactic acid increases of 2-5 mg./100 gm. or less and practically all samples possessed acid-associated flavors of lactic acid values of 10 mg./100 gm. However, the type of flavor, the temperature of incubation, and the type of organisms may somewhat influence these values.

Bacterial clump counts, in general, range from about 1.5 to 10 millions per ml. in those samples showing slight acid-associated flavors. The same factors which influence the acid flavor-lactic acid relationship are also of importance in this connection.

The methylene blue reduction time was markedly shortened in all samples of milk showing appreciable increases in lactic acid.

A general relationship was noted in the lactic acid increases obtained by the colorimetric lactic acid method and by titration. However, the titration results may vary appreciably from the true lactic acid changes in individual milks.

A discussion is given of the limitations and application of certain of these quality tests in a lactic acid grading program.

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# A TECHNIQUE FOR THE SIMULTANEOUS MEASUREMENT OF SEMEN QUALITY AND TESTIS HISTOLOGY IN VITAMIN A STUDIES OF THE DAIRY BULL\*

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Vitamin A deficiency is the most common of the known vitamin deficiencies which occur in cattle. The clinical symptoms, gross and microscopic pathology and therapy of vitamin A deficiency have been reported by many investigators and are not reviewed in this report.

It has long been known that vitamin A deficiency results in the cessation of spermatogenesis and atrophy of the testis in laboratory animals (8, 9) and in cattle recent studies (3, 4, 5, 6, 7) have shown vitamin A deficiency to have a similar effect on the testis of the bull.

When small numbers of animals are available it is obviously necessary to obtain as much information from each individual as possible. The standard procedures which have been developed for the measurement of semen quality are of unquestioned value but it is especially desirable to be able to determine the effects of vitamin A deficiency and therapy upon the spermatogenic tissue of the testis itself. This has formerly been possible by autopsy or by the surgical removal of one testis. Each of these methods has the common disadvantage of the limited number of times it can be applied to a particular animal. In recent years the development of biopsy techniques whereby small pieces of tissue can be removed at various intervals have made it possible to gather large amounts of data from single animals. Andrews and McKenzie (1) performed as many as 28 biopsies upon the genitalia of a single mare within a three-month period.

The purpose of this report is to describe the procedures used in the conduct of vitamin A studies in the dairy bull. The results obtained in this study should be regarded as preliminary in nature but it is believed that the method will be of value to other investigators in this field.

## EXPERIMENTAL PROCEDURES AND RESULTS

Semen was collected at weekly intervals using an artificial vagina. Two successive samples were obtained except in some cases when the bull refused to ejaculate twice. Semen examinations included volume of ejaculate, concentration and total numbers of spermatozoa, initial sperm motility, the maintenance of motility when the semen was stored at 40° F. and abnormal sperm forms. The details of the techniques have been published by Erb *et al.* (2). Testis tissue was removed by biopsy from the bull twice during

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the experiment. The testicular biopsies were performed by making a small incision in the lateral scrotal wall, exposing the tunica albuginea and, with a sharp scalpel, excising a testicular section about 5 mm. square. The testis usually bled freely but no special attempt to control bleeding was made and clotting occurred spontaneously. The tunica albuginea can, but need not, be sutured and one or two sutures can be placed in the scrotal wall. Recovery from the operation was uneventful in each case. The testicular tissue was immediately fixed in Bouin's fluid, dehydrated in an ethyl-butyl alcohol series, embedded in paraffin, sectioned and stained in Mallory's triple connective tissue stain.

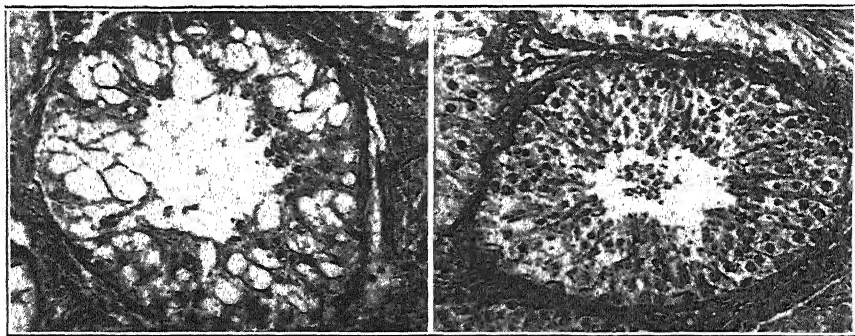


FIG. 1. The germinal epithelium has nearly completely disappeared and only a few spermatogonia are present.  $\times 220$ .

FIG. 2. The germinal epithelium has been greatly repaired and spermatozoa are present.

The bull used in this study was a crossbred Holstein-Guernsey born May 4, 1941. During the six-month period preceding the experiment, he was fed the standard Purdue growing ration supplemented with cod liver oil and was, to the best of our knowledge, a normal healthy animal. On June 8, 1942, the bull was placed on a vitamin-A-low ration composed of beet pulp as the only roughage and a grain mixture of white corn, oats, linseed oil meal, bonemeal and salt. On October 8, 1942, symptoms of advanced vitamin A deficiency were observed. The bull became blind, lost the coordination of movement and showed such gastro-intestinal disturbances as diarrhea and bloat. A testicular biopsy obtained from the right gonad on October 12 showed severe degeneration of the seminiferous tubules and the nearly complete disappearance of sperm from the lumina of the tubules (fig. 1). As summarized in table 1 semen quality decreased steadily until December 7, 1942.

Vitamin A therapy was begun on October 12, 1942, and the following treatment was given: October 12, 1942, to December 8, 1942, 60,000 I.U. of vitamin A daily; December 8, 1942, to January 5, 1943, 100,000 I.U. daily; January 5, 1943, to March 20, 1943, 150,000 I.U. daily.

A second section was removed from the right testis on February 5, 1943. Microscopic examination revealed that extensive but not complete repair of the seminiferous tubules had occurred (fig. 2). As shown in table 1 semen quality began a gradual improvement during the latter part of December. It can be observed that semen quality continued to decline after the initiation of vitamin A therapy on October 12, and that no spermatozoa were obtained in a series of 4 ejaculates collected during the interval ending

TABLE 1  
*Semen production of bull L. H. on a vitamin A low diet and after vitamin A therapy*

Interval ending	No. semen samples	Ave. vol.	Ave. sperm per cu. mm.	Ave. total sperm	Ave. initial sperm motility	Ave. sperm survival	Ave. abn. sperm per 1000
		cc.	thousand	million		days	
1942							
6-23	4	1.2	98	137	1.5	2.0	118
7-7	4	1.5	370	631	4.3	6.3	97
7-21	4	0.9	728	914	4.3	6.5	114
8-4	4	1.3	973	1244	4.8	14.0	101
8-18	4	1.1	793	961	4.0	7.0	97
9-1	4	0.7	913	585	3.8	6.0	55
9-14	4	0.4	435	230	3.0	4.0	88
9-29	4	1.0	730	834	2.0	4.3	132
10-12	4	1.0	220	229	1.3	1.0	408
10-26	4	1.4	20	27	0.5	0.5	.....
11-9	4	1.3	3	2	0	0	.....
11-24	4	1.5	0	0	0	0	.....
12-7	4	1.2	40	79	1.0	1.0	261
12-21	4						
1943							
1-5	4	1.3	83	124	0.8	0.8	189
1-18	4	1.1	288	386	2.3	2.3	243
2-1	6	1.5	762	1064	2.7	4.7	230
2-16	4	1.4	890	1380	1.0	2.8	267
3-1	4	1.9	245	745	1.0	2.8	350
3-16	4	1.9	98	245	2.0	1.5	159
3-22	4	0.5	115	108	2.0	1.8	142
4-7	4	2.4	683	1072	2.3	3.5	284

December 7. This can be theoretically explained as representing the gradual depletion of sperm stored in the vas deferens and epididymis. It can likewise be theorized that the lag in improvement of semen quality after the beginning of vitamin A treatment may have represented the time necessary for the restoration of the damaged germinal epithelium and the subsequent filling of the epididymis with newly formed spermatozoa.

It seems, therefore, that the effects of vitamin A deficiency and therapy upon spermatogenesis in the dairy bull can be effectively measured by the simultaneous study of the semen and the microscopic anatomy of the testis.

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# SEASONAL AND GEOGRAPHICAL VARIATIONS IN THE CITRIC ACID CONTENT OF DEFATTED MILK SOLIDS

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The literature on the citric acid content of milk shows a wide range in values. Allen (1) attributes the variations to the unreliable character of the methods of analysis and the analysis of samples which have undergone citric acid fermentation. He points out that the range reported is far out of proportion to the differences exhibited by the other constituents of milk.

Since the determination of the milk solids content of bread by means of its citric acid content has been suggested by Hartman and Hillig (5) and since the use of milk solids has been extended to a wide variety of other products, a study was made to determine the extent of seasonal and geographical variations in the citric acid content of defatted milk solids.

## REVIEW OF LITERATURE

The number of studies dealing with seasonal and geographical variations in milk is limited. Sherwood and Hammer (9) made 335 determinations on whole milk from individual cows and concluded: "There is no significant seasonal variation in the citric acid content of the milk." Their values ranged from 0.07 to 0.33 and averaged 0.18 per cent. Arup (2) analyzed 25 samples of fresh retail milk taken over a year's time. These values ranged from 0.150 to 0.206 per cent and did not indicate significant seasonal variation. He also determined the citric acid content of 11 samples of defatted milk solids of English, Irish, and American origin. There was no significant geographical variation, his results ranging from 0.151 to 0.184 per cent on the reconstituted basis.

A composite sample of 5 cows' milk on vitamin-C-free feed had a citric acid content of 0.08 per cent compared to 0.13 per cent citric acid in the milk from the same cows on pasture, according to Hess *et al.* (7). Supplee and Bellis (10) also found more citric acid in milk from cows on pasture (0.148%) than from cows on winter feed (0.142%).

Hartman and Hillig (4) conducted a collaborative study of the milk supply of 14 large cities located in various parts of this country and found that the average citric acid content of 58 samples was 0.16 per cent. These samples ranged from 0.14 to 0.19 per cent and exhibit some geographical variation, San Francisco showing the lowest and New Orleans the highest per cent citric acid.

Holm, Webb, and Deysher (8) studied the citric acid content of the milk from 4 cows throughout the lactation period. Their results indicated that

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TABLE 1

*Seasonal variation in citric acid content of defatted milk solids,  
1943 (moisture-free basis)*

Date	Citric acid (anhydrous)	Date	Citric acid (anhydrous)
	<i>per cent</i>		<i>per cent</i>
1/4	2.04	7/29	2.00
	2.05		1.98
1/16	2.02	8/2	1.95
	2.02		1.97
1/31	1.95	8/15	1.97
	1.95		1.95
2/11	2.00	8/20	1.95
	2.01		1.94
2/28	1.99	8/25	1.97
	1.98		1.99
3/9	2.00	9/15	2.01
	2.02		2.01
3/17	2.08	9/30	2.00
	2.09		1.99
4/9	2.09	10/20	1.98
	2.09		2.00
4/30	2.05	10/31	1.92
	2.06		1.92
5/12	2.04	11/11	1.89
	2.08		1.88
5/26	2.02	11/26	1.88
	2.04		1.88
6/15	2.06	12/9	1.95
	2.05		1.97
6/23	2.04	12/24	1.96
	2.04		1.97
7/15	2.00	<i>Average</i>	1.995

TABLE 2

*Geographical variations in citric acid content of defatted milk solids,  
July, 1943 (moisture-free basis)*

State	Citric acid (anhydrous)	State	Citric acid (anhydrous)
	<i>per cent</i>		<i>per cent</i>
Wisconsin .....	1.97	Mississippi .....	1.98
	1.97		1.99
Pennsylvania .....	2.05	Utah .....	1.89
	2.07		1.91
Minnesota .....	2.07	Michigan .....	1.84
	2.09		1.83
Missouri .....	2.01	Idaho .....	1.86
	2.02		1.90
Maryland .....	1.95	Oregon .....	1.98
	1.94		1.96
California .....	1.93	<i>Average</i> .....	1.961
	1.93		

there was a decrease in citric acid as the period of lactation progressed. Their values ranged from 0.19 to 0.29 per cent.

In a recent review of the literature, Hammer and Babel (3) concluded, "While the citric acid content of milk shows some variation, it appears that herd milk commonly contains from 0.16 to 0.18 per cent."

#### EXPERIMENTAL

The method used for the determination of citric acid in dry milk solids has been described in a previous publication (6). This method is based on the oxidation of the citrate ion by the perchlorate-cerate ion.

The samples of dry milk solids used in the seasonal variation study were collected from January, 1943, to January, 1944, at this plant. Each sample represents a composite of a large amount of milk. All determinations were made in duplicate.

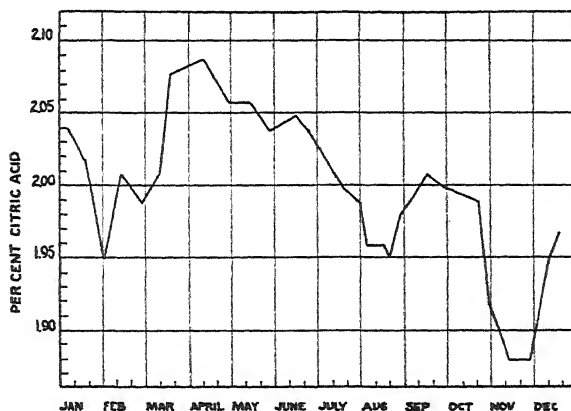


FIG. 1. Seasonal variation in citric acid content of defatted milk solids (moisture-free basis) during 1943.

The samples used to determine geographical variations were secured from the American Dry Milk Institute who received them during the month of July, 1943, from drying plants located in the specified states.

All samples were tested for moisture and lactic acid content and in no case did the latter exceed 0.01 per cent on the reconstituted basis. This indicates that the samples were of sufficiently good quality to preclude the possible loss of citric acid due to fermentation. The moisture was found to vary within the limits of 2.5 and 5.0 per cent, and for this reason, results were calculated to the moisture-free basis.

Figure 1 is a graphical representation of the data presented in table 1, showing the range in seasonal variation. Table 2 shows the geographical range in citric acid content of dry milk solids not fat.

## DISCUSSION

The study of the 27 samples taken throughout the year reveals that there is a small but definite seasonal variation in the citric acid content of defatted milk solids, ranging from a maximum 2.09 per cent in March and April to a minimum of 1.88 per cent in November and averaging 1.995 per cent. This does not substantiate the claim of Hess *et al.* and Supplee and Bellis that cows on pasture produce milk containing more citric acid than those on dry feed because a large majority of the cows in this section of the country were still on dry feed in March and April, 1943. These results seem to substantiate the data of Holm *et al.* which indicates that there is a decrease in the citric acid content of the milk as the period of lactation increases inasmuch as a large majority of the cows in this territory freshen in the spring. There are undoubtedly other factors, however, which are also concerned. One is that about 90 per cent of the herds supplying milk to this plant consist of Jersey cows. Another is that in 1943 there was an excessive amount of rainfall during April and May.

The study of the eleven samples taken from various parts of the country shows a range of values from 1.83 to 2.08 per cent and averaging 1.961 per cent. When calculated to a skim milk containing 9.3 per cent total solids, these figures are 0.165 to 0.193 per cent with an average of 0.182 per cent. There does not seem to be a relation between the section of the country in which the milk was produced and its citric acid content. For example, Minnesota has the highest and Michigan the lowest per cent citric acid. Nor is there any agreement between these figures and those of Hartman and Hillig (4). Furthermore, the difference in the range of values, when calculated on the reconstituted basis (0.028%), is not as great as the difference in the range reported by them (0.05%).

When calculated to a reconstituted basis, the range in citric acid in all samples—a total of 38—is 0.170 to 0.201 per cent (average 0.185%). This difference of 0.031 per cent is considerably less than would be expected in the light of previous work in which a difference as great as 0.26 per cent between maximum and minimum values is reported (9). The three most important reasons for this are: first, the use of a method which yielded close checks on duplicate samples; second, the analysis of samples which showed no evidence of fermentation; and third, the selection of samples which represented a composite of the milk from a large number of cows. As pointed out previously (6), the method used may give results slightly higher than the A.O.A.C. method.

It can be assumed, however, that the citric acid content of the defatted milk solids is sufficiently uniform throughout the country to be used as an index for the determination of the milk solids content of bread or other product.

## SUMMARY AND CONCLUSIONS

The citric acid content of 27 samples of defatted milk solids taken through the year 1943 varied from 1.88 to 2.09 per cent and averaged 1.995 per cent. The highest value occurred in March and April and the lowest in November, thus indicating a small but definite seasonal variation.

Samples of defatted milk solids taken from 11 different states during July, 1943, varied from 1.83 to 2.08 per cent (average 1.961%), thus indicating that there is a slight variation in the citric acid content of milk from different localities.

The citric acid content of defatted milk solids, however, is sufficiently uniform to be used as an index for the determination of the milk solids content of bread or other products.

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## FURTHER STUDIES ON BLOAT

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The purpose of this paper is to submit data, supplementing our earlier studies (1, 2, 3, 5), on the cause, production, and prevention of bloat.

### EXPERIMENTAL

Because several independent experiments are involved, most of the details on methods will be discussed under the separate sections. A few general statements can be made. Throughout the feeding period, when bloat was anticipated, the cows were constantly observed by at least one and usually two or more persons. We considered it safe to leave animals if bloat had not developed one hour after they were taken off pasture. Animals removed from the pasture and fed grain shortly thereafter would often bloat and occasionally had to be treated. In the series of experiments to be considered, there were 194 cases of bloat. Since the cattle were observed carefully, and were treated at once if seriously bloated, none was lost.

The experimental animals were mostly Holsteins and Jerseys, with two Guernseys and one Ayrshire. All are indicated by herd numbers through which the breed can be identified: those in the 1,000 series are Holsteins; 700 and 800 series, Jerseys; 600, Guernseys; 200, Ayrshires.

Bloat was determined in most instances by palpation of the left triangular area anterior to the hip and the degree of bloat was indicated by the terms "slight," "marked" or "severe." On the basis of a few instances in which the ruminal pressures were determined either directly with a mercury manometer or indirectly with an instrument devised by Kleiber (unpublished), the pressure in slight bloat was 10 to 23 mm. Hg. and in marked bloat 18 to 40 mm Hg. With one animal in evident distress and consequently designated as severely bloated, the pressure varied from 27 to 34 mm. Mg. In the normal relaxed rumen, the pressure is subatmospheric (2).

*The physical condition of the feed and the incidence of bloat.* According to our theory (2), bloat or tympany in cattle on green legumes usually results from a lack of sufficient coarse irritating fiber in the diet to elicit the eructation reflex. On the basis of that theory, bloat has been produced by depriving cows on immature alfalfa pasture of coarse roughage and prevented by supplementing such pasture with scabrous feeds such as Sudan grass hay or pasture (3). These results by no means prove the theory, however: conceivably, the presence or absence of coarse scratchy material in the diet of bloated cattle could be a mere coincidence. The present ex-

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periment was conducted to determine whether the physical condition of the feed does affect the incidence of bloat. Ground alfalfa hay and concentrates were compared with whole alfalfa hay and concentrates. Since the same feeds were used in approximately the same amounts and proportions, any difference in result should depend upon the physical rather than the chemical character of the alfalfa hay. A preliminary note describing the deficiencies associated with a diet of ground hay and concentrates has been published elsewhere (1).

Four lactating dairy cows, secreting 34 to 63 pounds of milk daily, were used. There were two experimental periods. In period 1 the cows received finely ground alfalfa hay plus concentrates. In period 2 the same cows received unground alfalfa hay from the same lot, together with concentrates. Period 1 was preceded by a 6-day preliminary period during which the animals were accustomed to the feed. The hay was ground to overcome the irritating effect of the coarse alfalfa stems upon the mucosa of the rumen.

Two slightly different rations were used in period 1. For the 6-day preliminary period and the first 9 days of the experiment the mixture was made up as follows: ground alfalfa hay prepared in a hammer mill using a No. 6 (6/64") screen, 60 per cent; rolled barley, 24 per cent; wheat bran, 10 per cent; soybean meal, 5 per cent; and NaCl, 1 per cent. For the following 6 days the mixture consisted of: ground alfalfa prepared as above, 50 per cent; finely ground barley, 39 per cent; wheat bran, 5 per cent; soybean meal, 5 per cent; and NaCl, 1 per cent. This change was made to determine whether a greater amount of concentrates in relation to ground hay would cause a higher incidence of bloat. Three of the four cows bloated on the first ration, and all bloated on the second. For palatability, these rations were moistened with an approximately equal weight of water just before feeding. Each animal received 7 pounds at the time of the morning milking (4:00 A.M.). Between 8:00 and 11:00 A.M. and again between 1:00 and 3:00 P.M., each was given all she would consume. Between 3:00 P.M. and 4:00 A.M. the cows received no food.

In the second period the system had to be modified somewhat, because one cannot feed concentrates and unground hay simultaneously and still maintain a definite proportion between them.

Had the feeding of roughage and concentrates separately been the only variable, bloat should have been more frequent during period 2 than in period 1. The rate of ruminal gas formation varies directly with the amount of feed consumed; further, the most rapid formation occurs during or shortly after eating (2). Since cattle eat concentrates more rapidly than roughage, the rapid ingestion of concentrates fed alone during period 2 favored bloat production. On diets limited to concentrates, bloat frequently occurs (6).



The concentrate mixture in period 2 was identical with that used during the first 9 days of period 1. Each cow received 3 pounds of concentrates at 4:00 A.M. At 8:00 A.M. enough more concentrates were fed to make up about half of the daily allowance. About 8:15 A.M., when the concentrates had been consumed, the animals were given all the unground alfalfa hay they would consume before 11:00 A.M. Between 1:00 and 2:30 P.M. they had enough hay to make their total daily allowance equal or slightly exceed the average individual consumption on the days during period 1 when they bloated. At 2:30 P.M. they were fed enough concentrates to maintain the same proportion of hay and concentrates as on the days when they bloated in period 1. Since cows 759 and 1003 bloated on both mixtures during period 1, the ratio of hay to concentrates for these animals in period 2 was midway between the two proportions. Cows 1028 and 1030 bloated chiefly on the 50 per cent hay-50 per cent concentrate ration; and thus this same ratio was maintained for these animals during period 2. The cows were

TABLE 1

*Bloat and daily feed consumption on ground alfalfa hay and concentrates as compared with alfalfa hay without grinding and concentrates*

Alfalfa hay	Cow 759		Cow 1003		Cow 1028		Cow 1030	
	Av. feed eaten, pounds	Times bloated*	Av. feed eaten, pounds	Times bloated	Av. feed eaten, pounds	Times bloated	Av. feed eaten, pounds	Times bloated
Ground .....	32.5	6	42.7	8	28.3	3	29.7	4
Unground .....	38.5	1	45.5	None	37.0	None	39.9	None

\* "Times bloated" refers to the number of days on which bloat occurred.

observed constantly for evidence of bloat between 8:00 A.M. and 12:00 noon and again between 1:00 and 5:00 P.M.

Table 1 shows the incidence of bloat and the average individual feed consumption. Twenty-one cases of slight bloat were encountered while the cows were receiving ground alfalfa, and only one case on whole alfalfa. Often, during period 1, gas under no appreciable pressure could be palpated; but these cases were not designated as bloat. As a rule, bloat occurred during or following the afternoon feeding and persisted 30 to 120 minutes. In four instances when bloat occurred in the morning, the same animals bloated again in the afternoon. This finding agrees with our previous experience in that bloat most often occurred during or after a period of eating, when the rumen was quite full.

One may ask why one case of bloat occurred on whole alfalfa and concentrates. Bloat occurs occasionally when large amounts of concentrates are fed. This animal, a Jersey, was a particularly greedy feeder for her size. She weighed 950 pounds as compared with an average of 1,480 pounds

for the other three animals, which were Holsteins. On the day in question she consumed 4 pounds more than on any day of period 1. On certain days we purposely increased the amount above the average for the days when bloat occurred during period 1. Not only was the whole alfalfa more palatable, but there was also less tendency for the animals to go off feed. Consequently the average consumption in period 2 was 6.9 pounds per day per animal greater than in period 1. Bloat most often occurs after the eating of large amounts; and thus, on the basis of feed consumed, more bloat would have been expected in period 2 than in period 1. It is difficult to escape the conclusion that the physical, rather than the chemical, character of the diet accounts for the greater frequency of bloat during period 1.

Although definite bloat could be produced by feeding ground alfalfa and concentrates, none of the resulting bloat cases was severe enough to require treatment. We cannot satisfactorily explain this fact. That the ground hay provided very little irritation seems certain, since only one or two feeble attempts to ruminate were observed when the cows were on the ground-alfalfa ration. As Schalk and Amadon (7) have shown, coarse roughage provides the stimulus for the regurgitation reflex. The cows ruminated normally when changed to whole alfalfa in period 2. The possibility that the rumen ingesta may have passed on to the omasum too rapidly for fatal bloat to occur is a tentative explanation. Had our studies been more prolonged, fatal bloat might have ensued, for Mead and Goss (6) reported it in cows limited to concentrates. Conceivably, fatal bloat in animals maintained on such diets for long periods may be due to ruminal atony, or it may have been that death was erroneously attributed to bloat. The animals had bloated frequently and were bloated when found dead but they were not actually observed in a state of tympany immediately preceding death.

*Bloat on machine-harvested alfalfa tops.* In the production of bloat, feeding green tops in the barn has certain advantages over pasturing: (A) The consumption of feed over definite intervals of the day can be more easily determined. (B) If one is interested in the rate of gas production, the feeding of tops to a confined animal has definite advantages. (C) In certain fields it may be impossible to produce bloat by pasturing because of contaminating weeds or grasses. If the weeds are lower than the alfalfa, they may be avoided by cutting the tops and feeding them in the barn. Animals pasturing on such fields, however, will often consume weeds as well as alfalfa; and thus, if the weeds are sufficiently coarse, bloat will be prevented.

For the first 5 days, four lactating cows were used; during the last 5 days, six lactating cows. In a preliminary period of 2 days the cows received grain only. During the first 5 days of the trial the cows received their regular daily allowance of concentrates (6-8 pounds) at 4:00 A.M. and

3:00 P.M.; during the last 5 days they received double their previous amounts of concentrates, fed in equal amounts at 4:00 and 7:45 A.M., 12:30 and 3:00 P.M. The cows had access to all the tops they would consume between 8:00 A.M. and 2:30 P.M. They were stanchioned during the feeding period and were turned out for water for a few minutes once in the morning and again once in the afternoon. For maximum consumption, the tops were fed in small amounts so that the animal received a fresh supply at approximately half-hour intervals. Severely bloated animals were treated with turpentine and deprived of further feed for the day.

The alfalfa tops were cut when the alfalfa was in a very succulent stage, 10 to 14 inches high. We attempted to cut tops about 4 inches long; but because of variations in plant growth they varied from about 2 to 8 inches. The tops were cut with an ordinary mower equipped with a metal apron for

TABLE 2

*Bloat on green alfalfa tops: total tops consumed, tops consumed when bloat was first manifest (figures in parenthesis), and maximum bloat reached during the day*

Date (1943)	Cow 1003	Cow 760	Cow 1030	Cow 788*
7-27	124 (116) slight	76	118	.....
7-28	64 (61) severe	43	79	.....
7-29	76 (76) severe	53	90 (43) slight	.....
7-30	52	33	75 (41) marked	.....
7-31	52	23	69	.....
8-24	78	40	73	47
8-25	86 (80) severe	86	117	55
8-26	86 (86) severe	69	88 (88) severe	31 (8) slight
8-27	61 (55) severe	71	15	60
8-28	67 (67) severe	44	27	39 (37) slight

\* No observations on this animal prior to 8-24.

catching the tops, the outer end of the sickle bar being held at the required height by means of a special shoe. One person followed the mower and raked the tops onto the apron.

Twelve cases of bloat were produced over the 10-day period, seven of which were severe enough to require treatment (table 2). Three cows failed to bloat. Because these three consumed similar amounts of feed, the data on only one (cow 760) are included in the table. The feed consumption of cows 760 and 788 (Jerseys) is considerably less than for the other two animals, which were Holsteins and therefore considerably larger. About one cow out of four bloated each day—that is, only about half the number we have usually encountered while pasturing alfalfa at a similar stage of development. The fact that certain individuals bloat with considerable regularity can be utilized to advantage in certain studies relating to bloat.

That bloat can be produced by feeding green tops is interesting because the feeding of cut green alfalfa in the corral has been successfully employed

in preventing bloat. Bloat is probably avoided by this procedure because the coarse lower stems are included and also because the alfalfa is usually cut at a more advanced stage. The fact that less bloat results from feeding tops than from pasturing may be explained by the possibility that coarser stems are cut than the cow would normally consume while pasturing.

Some tops were cut coincidentally with those fed green and dried in a fruit dehydrater. When these dry tops were fed to three cows over a 4-day period, only two slight cases of bloat occurred. The cows were given all the dry tops they would consume between 7:00 A.M. and 2:30 P.M. At 4:00 A.M., and again at 3:00 P.M. each cow received 4 pounds of concentrates. Drying of the tops apparently reduced their capacity for producing bloat.

TABLE 3  
*Feeding of barley straw to prevent bloat on alfalfa pasture*

Date (Oct., 1943)	Concentrates per cow, pounds	Straw*		Number of cows	Number cases of bloat
		Fed per cow, pounds	Av. amount consumed, pounds		
4	6†	.....	.....	8	4
5	6†	.....	.....	8	5
6	6†	.....	.....	8	5
7	.....	.....	.....	8	3
8	.....	5	1.7	8	4
9	.....	5	2.2	8	4
10	.....	8	5.0	7	3
11	.....	8	4.1	7	4
12	.....	.....	.....	8	6
13	.....	.....	.....	8	5
14	.....	.....	.....	8	6
15	6‡	.....	.....	8	3
16	6‡	.....	.....	8	6

\* When 5 pounds of straw were fed per cow, this amount was given 2 hours prior to pasturing on alfalfa; when 8 pounds were given, the cows had access to the straw overnight.

† Concentrates fed afternoon before day of trial.

‡ Concentrates fed on morning of trial rather than night before.

This was not because of lowered feed consumption: the total dry matter ingested was about the same as when green tops were fed. Probably the dry tops were more irritating, but more rumination occurred on both green and dry tops than one would expect if the same field, from which the tops were cut, were to be pastured. At any rate, these preliminary experiments do not indicate that dry tops are satisfactory for producing bloat.

*Feeding of barley straw to prevent bloat.* Earlier studies (3) indicated that bloat on alfalfa pasture could be prevented by supplementing with Sudan grass hay or pasture. Alfalfa hay varied in effectiveness, presumably according to its coarseness. Since straw is available in most regions at a low cost, its effectiveness in preventing bloat was studied. Four lactating and three or four dry dairy cows were used. All were treated similarly

except that the dry cows were fed straw as a group, whereas the lactating cows were fed individually. Table 3 shows details of the feeding and the frequency of bloat. Obviously, straw did not prevent bloat under the conditions of this experiment. One cow bloated after consuming 5.5 pounds of straw the night before. There may have been a very slight decrease in the incidence of bloat; but three animals bloated severely and required treatment while receiving straw. A tentative explanation of the ineffectiveness of straw is the rather small amount consumed. As the consumption of Sudan grass hay was not measured in our earlier studies (3), we do not know the actual differences in amounts of Sudan grass hay and straw con-

TABLE 4

*Variations in individual susceptibility to bloat of cows 1003, 760, and 1030, as compared with the incidence of bloat for the group as a whole*

Key: D, definitely slightly bloated; M, markedly bloated; S, severely bloated

Date (1943)	Num- ber cows in trial	Num- ber cows bloat- ed	Cow 1003	Cow 760	Cow 1030	Date (1943)	Num- ber cows in trial	Num- ber cows bloat- ed	Cow 1003	Cow 760	Cow 1030
7-27	4	1	D	.....	.....	9-23	10	2	.....	.....	D
7-28	4	1	S	.....	.....	9-24	10	3	D	.....	.....
7-29	4	2	S	.....	D	9-27	10	3	D	.....	.....
7-30	4	1	.....	.....	M	9-30	10	4	M	.....	.....
7-31	4	0	.....	.....	.....	10-1	10	5	D	.....	.....
8-24	6	0	.....	.....	.....	10-2	10	3	.....	.....	.....
8-25	6	1	S	.....	.....	10-3	10	2	.....	.....	.....
8-26	6	3	S	.....	S	10-4	8	4	M	.....	.....
8-27	6	1	S	.....	.....	10-5	8	5	M	.....	.....
8-28	6	2	S	.....	.....	10-6	8	5	M	M	.....
9-13	29	6	.....	.....	.....	10-7	8	3	M	.....	.....
9-14	28	9	D	.....	M	10-8	8	4	M	.....	D
9-15	27	12	M	Out*	M	10-9	8	4	D	.....	D
9-16	28	9	.....	M	D	10-10	7	3	M	.....	.....
9-17	28	11	M	D	D	10-11	7	4	D	.....	D
9-18	13	2	.....	.....	.....	10-12	8	6	D	.....	D
9-19	10	2	.....	D	.....	10-13	8	5	D	M	M
9-20	10	3	S	.....	D	10-14	8	6	D	M	D
9-21	10	4	S	D	.....	10-15	8	3	.....	M	.....
9-22	10	2	.....	.....	.....	10-16	8	6	D	M	D

\* "Out" means not in trial on this date.

sumed. We are basing our explanation, therefore, on the well-recognized fact that barley straw is rather unpalatable.

*Variations in individual susceptibility.* As already reported (3), individuals appear to differ markedly in susceptibility to bloat. Although more extensive observations have confirmed this belief, clearly too, an individual may vary in susceptibility. In other words, animals hitherto rather resistant suddenly become very susceptible. As examples, data on individual susceptibility of cows 1003, 760, and 1030 are shown in table 4. The number of animals in the trial and the total number bloating each day are also shown

in order that the results may be better evaluated. Cow 1003 bloated more regularly than the other three animals shown in the table. Severe bloat, however, occurred in her most regularly between July 27 and August 28, when the incidence of bloat for all the animals on test was low. Between October 11 and 16 she bloated only slightly even though the incidence of bloat for the group was high. Cows 760 and 1030 were very irregular. Cow 760 did not bloat for 12 days beginning July 27, whereas beginning October 13 she was markedly bloated on four consecutive days. Though some of these variations undoubtedly result from variations in appetite, this explanation does not suffice for all cases. We have data on food consumption for cow 760 during part of both periods mentioned. The average consumption of green tops over the first 2 hours of the feeding period on August 24 to August 28 inclusive was 40 pounds. In addition, this animal received 14 pounds of concentrates per day. Her average consumption for the first 2 hours of the feeding period on October 13 to 16 inclusive was 35 pounds. On October 13 and 14 she received no grain, and on the following 2 days she received 6 pounds shortly before being turned out to pasture. Actually, therefore, she consumed more during the first 2 hours of the period when she did not bloat than during the last period, when she bloated each day. Her grain consumption, too, was greater during this first period. To be sure, we have considered only the amount of alfalfa consumed during the first 2 hours of the feeding period; but in our experience this is usually a reliable criterion in comparing food intake on different days.

If feed consumption does not explain all the variations in individual susceptibility, what is responsible? Although our data give no clue, we suspect that variations in ruminal motility may be involved. As we have pointed out earlier (2), belching occurs only when the rumen is in an active state of contraction. Apparently a lack of coarse roughage for a considerable period may lead to faulty ruminal contractions.

*Time at which bloating occurs.* In earlier experiments (3), a majority of animals bloated 1.5 to 3.5 hours after being turned onto pasture. Although further experiments conducted under similar conditions have confirmed this result, we now find that the time of bloating varies considerably according to the feeding regime. In the ground hay and concentrate experiment cited above, all but 4 of the 21 cases of bloat occurred after 1:30 P.M. even though feeding began at 4:00 A.M.

Table 5 shows how supplemental feeding affects the interval between the time the cows are turned into alfalfa pasture and the occurrence of bloat. The feeding of grain just before pasturing greatly reduced the interval before bloat was manifest. Our data do not indicate, however, that this procedure increased either the number of cows bloating or the severity of bloat.

*Susceptibility of dry and lactating cows.* For comparison of susceptibility, 11 dry and 17 lactating cows were turned into the same field. There

were 9 cases of bloat over the 4-day period (20.5 per cent) among the dry cows as compared with 32 cases of bloat for the lactating cows (47.0 per cent). These data are in line with what one would expect on the basis of feed consumption—the lactating cows eating considerably more, as we shall show later. There also appears to be a tendency for lactating cows to bloat more severely.

*Amount of green alfalfa consumed.* Table 2 shows the consumption of green alfalfa tops in the barn. Studies were also made on the consumption

TABLE 5

*The relation of supplemental feeding to the time (in minutes) for bloat to occur after cattle are turned into alfalfa pasture*

Date (Oct., 1943)	Supplemental feeding	Cow 597	Cow 738*	Cow 757	Cow 1028	Cow 1046	Cow 788	Cow 760	Cow 1003	Cow 1030	Aver- age
4	Grain afternoon before	..... †	75	.....	65	275	212	.....	85	.....	142
5	Grain afternoon before	.....	.....	430	75	85	75	.....	439	.....	221
6	Grain afternoon before	.....	.....	.....	345	45	65	170	352	.....	195
7	None	.....	331	.....	143	.....	103	.....	331	.....	227
8	Straw night be- fore	.....	.....	.....	140	.....	175	.....	360	285	240
9	Straw night be- fore	.....	153	.....	122	.....	63	.....	326	301	193
10	Straw night be- fore	.....	287	363	115	.....	68	.....	328	.....	232
11	Straw night be- fore	.....	105	350	93	.....	100	.....	410	410	245
12	None	313	.....	.....	71	105	88	.....	388	293	210
13	None	.....	133	.....	93	.....	143	163	333	247	185
14	None	.....	104	.....	58	91	51	73	371	186	133
15	Grain 1 hour before	.....	97	.....	46	.....	71	46	.....	.....	65
16	Grain 1 hour before	140	28	.....	.....	38	28	33	106	106	68

\* This cow received no straw at any time, and cow 1028 received no straw on October 10 and 11.

† Blank spaces indicate that no bloat occurred.

of alfalfa during the first 2 hours of pasturing. The cows were weighed before and after pasturing, and all voided urine and feces were collected and weighed. Insensible losses were not taken into account. As the period of pasturing extended from 8:00 A.M. to 10:00 A.M., there were also some variations in dew. In samples taken on one representative day the per cent dry matter of the alfalfa ranged from 15.8 at 8:10 to 19.5 at 10:15 A.M. We are giving these figures because variation in moisture is presumably the major source of error. As the animals from which data are cited were quiet and well trained, there was no appreciable error due to failure in collecting all excreta. We found, however, that very few of the other animals at our

disposal were so gentle as might be desired, particularly for the collection of feces. The animals were allowed to graze when and where they chose in the field; but a halter with a long lead rope restrained them when excreta were being weighed and recorded.

The amount of alfalfa consumed during the first 2 hours of pasturing is shown in table 6 for one dry and one lactating cow under several different regimes of supplemental feeding. The average initial weight before pasturing for the 13 days of the experiment was 824 pounds for cow 757 and

TABLE 6

*Amount of green alfalfa consumed by a dry and a lactating cow over a two-hour period and its relation to supplemental feeding*

Date (1943)	Supplement fed	Alfalfa consumed, in pounds	
		Cow 757 (dry)	Cow 760 (lactating)
10-7	None	17.0	43.5
10-12	None	19.7	53.8
10-13	None	19.5	50.6*
10-14	None	16.2	30.8†
	Average	18.1	44.7
10-5	6 pounds concentrates the night before	12.4‡	49.6
10-6	6 pounds concentrates the night before	12.5	39.3*
	Average	12.5	44.5
10-15	6 pounds concentrates in morning	15.2	29.2‡
10-16	6 pounds concentrates in morning	3.0§	28.8†
	Average	9.1	29.0
10-8	5 pounds straw at 6:30 A.M.	35.2	60.2
10-9	5 pounds straw at 6:30 A.M.	25.8	50.0
10-10	8 pounds straw overnight	22.4‡	36.5
10-11	8 pounds straw overnight	33.8‡	43.6
	Average	29.3	47.6

\* Markedly bloated at 11:10 A.M.

† Bloated during more than half of feed consumption period.

‡ Slightly bloated at 3:00 P.M.

§ Ruminal gas could be palpated during all except first 28 minutes of pasturing period, but not under sufficient pressure to call "slight bloat"; this gas no doubt depressed appetite.

909 pounds for cow 760. Assuming that the feed consumption increases in proportion to  $\frac{3}{4}$  power of body weight, cow 760 would consume 8 per cent more than cow 757. In comparing feed consumption of lactating and dry cows, one should preferably have several animals in each group. That was our intention, but (as mentioned) it was difficult to find cooperative animals. As an indication of relative capacity, the fat records of these two animals as senior yearlings were 345 pounds for cow 760 and 447 pounds for cow 757. Without supplemental feeding, the lactating animal ate  $2\frac{1}{2}$  times more than the other. The feeding of straw just before pasturing increased feed consumption. Although the straw did not prevent bloat, it apparently had a tendency during the first hour or two to prevent the slight accumulation



of gas (really an early stage of bloat) that depresses appetite. Individuals react quite differently to gas accumulation. Many will stop eating the moment gas can be palpated, whereas others will continue eating even though the gas is under considerable pressure. These latter animals are more liable to bloat seriously. The fact that alfalfa consumption is actually stimulated by prior feeding of straw may partly explain why it is not more efficient in reducing the incidence of bloat.

The feeding of concentrates just before pasturing had the opposite effect from feeding straw: the consumption of alfalfa was reduced, largely because bloat depressed appetite. When concentrates were fed the night before pasturing, the feed consumption was comparable with that when no concentrates were fed.<sup>1</sup>

As already mentioned, the rate of consumption of alfalfa by pasturing was comparable with the consumption of green tops in the barn over a 2-hour period. Probably, then, the daily consumption of tops in the barn as shown in table 2 gives a fair index for similar periods of time in the field. We have data on one dry cow pastured 6 hours daily for 3 days. Her average initial weight before pasturing was 1,079 pounds, and she consumed an average of 33.7 pounds daily over the 6-hour period. This cow received no supplemental feeding. Comparing this figure with the figures for cow 757 (table 6), which received no supplements, one could estimate that a dry cow eats as much during the first 2 hours as during the next 4. Since, however, different cows and different fields are involved, the estimate is of doubtful value.

A point of primary interest here is the amount of alfalfa consumed before bloating occurs. Table 2 gives some data when green alfalfa tops were fed. As a rule, bloating did not occur until more than 50 pounds had been consumed; but one animal did bloat after consuming 8 pounds. Table 6 gives limited data for cows on pasture. If grain is fed just before pasturing, bloat may take place before much alfalfa is consumed. The variable time at which bloat occurs after cows are turned into pasture (table 5) indicates that there is considerable latitude in the amount consumed before bloating. Assuming that the period of time on pasture before bloat occurs is an index of consumption, there are marked individual differences in this respect. For example, compare cows 788 and 1003 in table 5. The former usually bloated before the end of the second hour of pasturing, whereas No. 1003 usually did not bloat until after the fifth hour even though she was a particularly good feeder (see tables 1 and 2 for feed-consumption data).

<sup>1</sup> We have not ascertained how the feeding of concentrates before pasturing affects the incidence of bloat. Without submitting data in confirmation, Espe, Jacobson, and Cannon (4) state, "Feeding grain, dry hay or silage before putting cattle on legume pastures will help prevent over-eating and, in turn, bloat." The amounts of grain we have used have had no appreciable effect on the incidence of bloat (table 3).

## DISCUSSION

These studies support the view that the lack of sufficient coarse irritating material in the diet is the chief cause of bloat on legume pastures. The work on ground alfalfa hay gives clear-cut evidence that the physical character of the feed is an important etiological factor in bloat. Additional evidence is provided by the fact that we have been able to predict bloat on eight different fields, some of them on two or three occasions, merely by taking into account the succulence of the alfalfa and the absence of weeds or grasses. We did fail to produce bloat in one field early in the spring even though we were successful in two other attempts. When pastured early, this field was contaminated with annual grasses. Since the grasses were lower than the alfalfa, we expected that the cows would eat only the alfalfa. On the contrary, they consumed grasses for the most part.

The evidence indicates, further, that lactating cows are more subject to bloat because of greater feed consumption. Reviewing the evidence submitted in table 2, however, one sees that bloat is not invariably associated with high feed consumption. Because of variations in individual susceptibility, not correlated with feed consumption or with the bloat-producing capacity of the feed, these appear not to be the only factors that determine whether or not a certain individual will bloat on a given day. As we have already suggested, variations in ruminal motility may account for these changes in susceptibility.

Bloat has been produced by feeding ground hay and concentrates or by feeding alfalfa tops in the barn as well as by pasturing.<sup>2</sup> We can add somewhat to our previous discussion (3) on means of inducing bloat by pasturing legumes. Very young alfalfa (4-6") for instance, is rather unpalatable and thus not suitable for bloat studies. Cattle turned into young alfalfa will invariably consume the less succulent alfalfa (more stemmy) on the ridges used to check irrigation water. On the other hand, alfalfa is no longer suitable for bloat studies when the top part of the plant becomes stemmy. Apparently one reason why bloat is produced rather easily under our conditions is that we can supply the alfalfa with optimal amounts of water throughout the summer. In late fall a field may remain in a bloat-producing stage for several weeks because of the slow maturity resulting from cooler weather.

## SUMMARY AND CONCLUSIONS

1. In a series of experiments involving an average of 9 cows for 61 days, 194 cases of bloat were produced.

2. Demonstrating that the physical character of the feed is an important etiological factor in bloat, 21 mild cases of bloat were produced in 4 cows over

<sup>2</sup> The effect of feeding green alfalfa tops to ewes in the barn has also been studied (unpublished data). Over an 11-day period 26 cases of bloat were encountered in 5 ewes; but none of the cases was severe enough to require treatment.

a 15-day period by feeding ground alfalfa hay and concentrates in a 60-40 or 50-50 ratio. Only one case of bloat occurred when concentrates were fed in the same proportion with the same hay unground.

3. Twelve cases of bloat, 7 of which were severe enough to require treatment, were induced by feeding green alfalfa tops in the barn to 4-6 cows for 9 days.

4. Supplemental feeding of barley straw at night to cows pastured on alfalfa during the day was not effective in preventing bloat.

5. Variations in individual susceptibility occurred that could not be explained either by feed consumption or by the nature of the feed. We conclude that these changes in susceptibility of an individual depend on some change in physiological activity of the rumen—for example, motility.

6. Bloat occurred sooner after animals were turned into pasture if grain was fed just before pasturing.

7. Certain data indicate that lactating cows are more susceptible than dry cows.

8. Over a 12-day period, the average amount of alfalfa consumed by a dry cow during the first two hours of pasturing was 19.4 pounds as compared with 43 pounds for a lactating cow. The feeding of straw overnight, before pasturing, markedly increased the consumption of alfalfa by the dry cow during the first two hours; the affect on the lactating cow was less conspicuous. Feeding of concentrates just before pasturing decreased consumption of alfalfa, with no apparent effect upon the incidence or severity of bloat.

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## NON-PERMEABILITY OF THE MAMMARY GLAND TO THYROID HORMONE

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As a result of experiments first reported by Graham (10, 11) and repeatedly confirmed by a number of investigators, it is now well established that increases in both the milk yield and butterfat test of lactating cows can be brought about by the administration of thyroid substance or thyroxine. Practical use of thyroid for this purpose was prevented by its limited supply and high cost.

However, detailed studies of the authors on the formation of thyroidally active compounds by combining iodine with casein (25, 18, 20, 26) finally resulted in the development of an inexpensive process for the formation of a synthetic "thyroprotein" that exerts many times the effect of U.S.P. thyroid, as measured by the usual assay methods. Proof that the active principal in this synthetic thyroprotein is identical with that in natural thyroid was provided by the recovery in high yield of crystalline d,l-thyroxine (21) and, finally, crystalline l-thyroxine (22).

Pronounced increases in daily milk yield and milk fat percentage were observed when synthetic thyroprotein was fed to lactating cows, as reported by Reineke and Turner (23) and Reineke (19), and confirmed by Van Landingham, Henderson and Weakly (34), and Blaxter (3).

Although much more extensive experiments will be required, particularly on the effects of continuous feeding of thyroprotein to dairy cattle, before its effects can be fully evaluated this product holds considerable promise as a means of increasing and maintaining milk production.

The question then arises whether or not the feeding of thyroprotein would result in transmission of thyroxine into the milk.

Although a few experiments have been interpreted to indicate the passage of thyroid hormone into the milk, no direct evidence in support of such a view has been reported. Having noted that experimental hyperthyreosis reduced the growth rate of rats, Lukaes (16) administered thyroid to lactating rats and observed the growth rate of the young. He reported that the young of the hyperthyroid females were retarded in their growth 31 to 34 per cent. From this he concluded that thyroid hormone passes into the milk and further that the amount present changes according to the concentration in the mother's system. However, it is known that both excessive hyperthyreosis (Herman, Graham and Turner, 13) and thyroidectomy (Folley, 8,

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Preheim, 17) will depress the rate of milk secretion. Preheim has presented a good review of the literature.

It appears from an examination of all the experiments in this field that lactation is suppressed by hypothyroidism, stimulated increasingly by higher levels of thyroid secretion, and is again depressed when the stimulation reaches excessive levels. Since both thyroidectomy and hyperthyreosis depress milk secretion, the growth rate of nursing young is too nonspecific to determine whether or not thyroprotein is transmitted to the milk.

Konsulov (15) reported that thyroidin administered to a lactating mother passed into the milk and caused an increase in the carbon dioxide production of the nursing infant. This work must be interpreted with caution, however, since Brody (4) found that the metabolism of rats receiving larger amounts of milk (by reducing the number in a litter) have a markedly higher rate of oxygen consumption than those receiving less milk. Thus following thyroid administration, a higher rate of milk secretion would increase the metabolism of the young due to greater intake of nutrients, without postulating the passage of thyroid into the milk.

In all cases where a more critical and specific measure of the possible transference of thyroid hormone from the maternal system to the milk has been made, the results have been completely negative. Simpson (31) compared the growth rate of twin lambs and kids, one member of each pair being thyroidectomized at about one month of age and the other serving as a normal control. The thyroidectomized animals soon showed symptoms of thyroid deficiency even though the food consisted entirely of the mother's milk for some weeks. He concluded that these facts do not support the idea that a thyroid hormone is present in mother's milk, at any rate in quantity sufficient to supply the needs of the athyroid offspring.

Dorff (6) made similar observations in man in the case of twins, one of which was cretinous. Previous to this, Herman (14) and Gordon (9) noted cases of cretinism which were not improved by nursing, but responded to thyroid treatment. Siegert (30) reported that in cretins with a complete absence of the thyroid gland signs of deficiency may be apparent at birth or shortly thereafter.

In the experiments of Reineke and Turner (24) with thyroidectomized kids and Brody and Frankenbach (5) with thyroidectomized calves all of the thyroidectomized animals rapidly developed cretinism even though they were fed milk from normal animals with intact thyroids.

Earlier experiments in which young mammals were thyroidectomized and continued on normal milk without curative effect on the induced cretinism were reported by Simpson (32) on sheep, Basinger (1) on rabbits, Binswanger (2) on dogs and Salmon (28) on rats.

These extensive experiments on a long series of mammals from the rat to the cow uniformly fail to provide evidence of the transfer of thyroid hormone to the milk of animals with a normal thyroid gland.

It is also reported by Elmer and Rychlik (7) that milk yields only a trace of thyroxine-like fraction after hydrolysis. In his book, "The Endocrine Function of Iodine," Salter (29) reviewed the evidence on the transference of thyroxine through the milk and concluded that "if present at all it must be exceedingly small."

Even though the evidence fails to show a single instance for the passage of normal thyroid secretion into the milk the possibility was suggested that when thyroidal substances are fed continuously the thyroid level of the blood might be elevated sufficiently to permit passage of traces of active substance into the milk. In view of the possible use of the new synthetic thyroprotein for the stimulation of increased lactation, and the ultimate consumption of the milk so produced by humans, it seemed desirable to investigate further the question whether thyroprotein-fed cows would secrete detectable amounts of this hormone in their milk.

#### EXPERIMENTAL

Three experiments were conducted in which synthetic thyroprotein was fed to cows, and the milk was fed to suitable test animals for detection of possible thyroidal properties. The synthetic thyroprotein, hereafter designated as "Protamone," was kindly supplied by Dr. W. R. Graham, Jr., Cerophyl Laboratories, Kansas City, Missouri. In experiments number I and II the experimental milk was fed to guinea pigs and in experiment III young thyroidectomized goats were used as test animals. In experiment IV similar milk was tested in man.

*Experiments with guinea pigs.* Two groups of six mature guinea pigs, paired as to body weight and general condition, were selected for experiment I. Each animal was numbered and placed in an individual cage and as a preliminary, both groups were fed normal pasteurized milk for a period of two weeks to accustom them to this type of diet. Each animal was given 100 ml. of milk daily, and the amount refused was measured back, the actual milk consumption thus being estimated by difference. To provide additional nutritive factors each animal was given 6 grams of dehydrated and finely ground cereal grass<sup>1</sup> daily which was mixed with 25 ml. of milk. A mineral supplement consisting of 1 mg. of iron, as ferric pyro-phosphate; 0.1 mg. of copper, as copper sulfate; and 0.1 mg. of manganese, as manganese sulfate, was fed daily in a gelatin capsule. In addition to the milk-consumption data, the body weight of each animal was recorded daily. One animal in each group failed to do well after 10 days on the normal milk diet so new animals were substituted for each.

Metabolism measurements of the individual guinea pigs on the experiment were made during the last three days of the preliminary period and

<sup>1</sup> Cerogras, kindly supplied by the Cerophyl Laboratories, Inc., 2438 Broadway, Kansas City, Missouri.

at intervals for fourteen days after being placed on the milk from the thyroprotein-fed cow. The determinations were made in a modified Haldane type, open circuit respiration apparatus for small animals, using the weight of carbon dioxide produced during a period of one hour as an indirect measure of the metabolism. To facilitate comparisons of values for individual animals the data were converted to the basis of carbon dioxide production per 100 gm. body weight per hour.

After 14 days on the milk diet, raw milk from normal Holsteins and from Holsteins on a high dosage of thyroprotein was substituted in the diets of groups I and II, respectively.

Rather extensive feeding trials with synthetic thyroprotein of the potency now available (3 to 4 per cent as potent as d,l-thyroxine) have shown that 1 to 1½ grams per 100 lbs. body weight daily is tolerated nicely by cows and will cause significant increases in their production of milk and butterfat. In order to make the present test as critical as possible, the cow supplying the milk for this experiment was given the maximum amount of thyroprotein that she would tolerate. Beginning on March 18, 1943, she was fed 90 grams of thyroprotein daily in her grain feed, an amount far above what would ever be used in practice. By April 7, it was evident from the cow's physical symptoms that she would not tolerate this dosage indefinitely. Accordingly the dosage was reduced to 45 grams daily, and continued at this level to the end of the experiment. The guinea pigs in group II received milk from this cow from April 1 to April 14, 1943.

#### RESULTS

Inasmuch as there were no significant differences in the milk consumption or trend of body weight the detailed data on these factors will be omitted for the sake of brevity. After the guinea pigs had become accustomed to the milk diet they consumed an average of 80 to 90 ml. of fluid milk daily. In addition they received approximately 25 ml. daily with the grass supplement, making a total consumption of 100 ml. or more daily.

After an initial drop in body weight during the preliminary period while the animals were becoming accustomed to the milk diet there was some gain in weight, and then the values leveled out to a uniform figure. The milk from the "Protamone-fed" cow had no effect on body weight of guinea pigs as compared to the controls on normal milk.

A comparison of the average carbon dioxide output of the two groups of guinea pigs, determined at intervals during the experiment, is shown graphically in figure 1. With the exception of the minor fluctuations that are always encountered in metabolism studies, the average values for both groups remained at practically the same level throughout the experiment. Therefore, no evidence could be found for the passage of thyroidal substance into the milk of "Protamone-fed" cows during the period covered in this experiment.



All of the earlier clinical and experimental work on thyroid administration indicates that the dosage does not become cumulative over a long period of time. Instead there is a gradual rise in metabolism, starting about the third day after dosage is begun, and reaching a peak in about two weeks. The metabolism then remains constant at this new level as long as the dosage is continued. Nevertheless, it seemed desirable to extend the experiments on milk from "Protamone-fed" cows over a longer period to determine whether any hormone would be transmitted to the milk with continuous feeding.

Therefore, experiment II was set up using the same experimental procedure described for experiment I. However, the period during which the experimental milk was fed was extended to 8 weeks. During the last 2

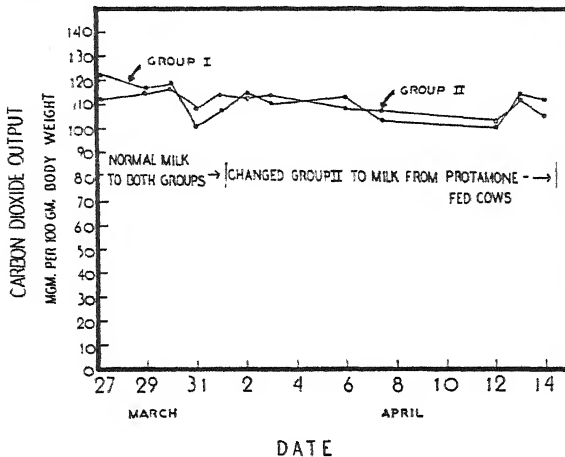


FIG. 1. The effect of milk from a "Protamone-fed" cow on the resting carbon dioxide output of mature guinea pigs. Group I received normal milk throughout. During the last two weeks group II received milk from a cow that was being fed a high dosage of "Protamone."

weeks the animals in group I (receiving milk from a "Protamone-fed" cow) were injected with l-thyroxine at the rate of 2.5 micrograms per 100 gm. body weight daily. Group II received normal Holstein milk throughout. Milk for the experimental group was obtained from a Holstein cow receiving the more nearly optimal dosage of  $1\frac{1}{2}$  gm. "Protamone" per 100 lbs. body weight. On this dosage this cow showed an initial rise of 11 per cent in milk production, but no serious losses in body weight. Two groups of 6 guinea pigs were started on experiment, but 1 animal of each group failed to become adapted to the milk diet, and, therefore, data are reported on 5 animals in each group.

The milk consumption and body weights of both groups followed the same trend as described in the previous experiments. Therefore, no further comment will be made on these points.

A comparison of the average carbon dioxide output of the two groups of guinea pigs is shown graphically in figure 2. Here again both groups follow the same metabolic trend with minor deviations, throughout the six weeks on the experimental milk diet. Analysis of the individual determinations during the six-week period before group I was injected with thyroxine revealed that 95 determinations of the carbon dioxide output of the animals in group I averaged 106.5 mg. per 100 gm. body weight, standard deviation 11.803 mg. The average carbon dioxide output in 88 determinations on group II during the same period was 102.8 mg. per 100 gm. body weight, standard deviation 14.051 mg. The standard error of the difference was 6.0676 mg., *t* value, 0.576. As shown by the "t" test (Snedecor, 33) the difference between the two groups is insignificant. By comparison with

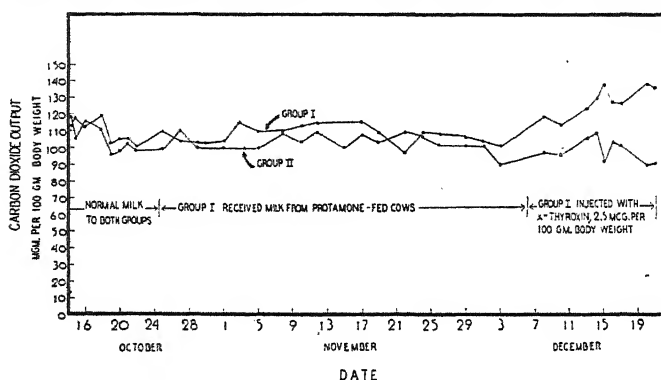


Fig. 2. The effect of milk from a "Protamone-fed" cow on the resting carbon dioxide output of mature guinea pigs. During the six-week period that group I received the experimental milk no significant elevation of metabolism was observed. Injection of 2.5 micrograms of l-thyroxine during the final period caused a 25 per cent increase in metabolic rate.

these values obtained on a milk diet, 110 determinations of the resting carbon dioxide of normal guinea pigs on a stock diet as determined previously (unpublished) was 105.7 mg. per 100 gm. per hour. Thus it is apparent that the guinea pigs fed the experimental milk in the maximum amount they would consume did not differ significantly in their metabolism from similar animals on a normal milk diet, or on a stock diet. No evidence could be found, therefore, for the transmission of thyroidal substance to the milk of cows fed "Protamone."

It is well known that the guinea pig is the most responsive to thyroidal stimulation of all the laboratory animals, and, therefore, should be capable of detecting such transference if it occurs in biologically significant amounts. As an indication of their sensitivity, attention is drawn to the results obtained (fig. 2) when 2.5 micrograms of l-thyroxine per 100 gm. body weight was injected daily in the animals of group I. The average carbon dioxide

output of this group for six days previous to the injections was 105.4 mg. per 100 gm. body weight. The carbon dioxide output began to rise by the third day of injection and reached the maximum by about the eighth day. The carbon dioxide output during the last six days on which determinations were made was 132.5 mg. per 100 gm. body weight, an increase of 25.7 per cent.

*Experiment with thyroidectomized goats.* In addition to the experiments with guinea pigs, milk from "Protamone-fed" cows was fed to a group of thyroidectomized goats in order to test possible thyroidal effects.

Thyroidectomized animals are very sensitive to thyroidal substance since

TABLE 1

*The metabolism of thyroidectomized goats before and after receiving milk from thyroprotein-fed cows*

Date	Metabolism, calories per day					Remarks
	Goat No. 40	Goat No. 26	Goat No. 57	Goat No. 19	Average all goats	
3/1/43	727	1024	936	947	924	Preliminary period
3/2/43	669	1026	982	837		
3/3/43	808	1007	852	1018		
3/4/43	882	926	806	806		
3/5/43	806	1192	1236	994		
Mean	778	1035	962	920	924	
3/9/43	.....	1247	1312	1010	918	Each goat consumed 5 lbs. milk from thyroprotein-fed cows daily
3/10/43	969	1245	1068	958		
3/11/43	985	1219	743	942		
3/12/43	619	716	1184	814		
3/16/43	773	1002	926	1144		
3/18/43	587	1119	909	798		
3/19/43	811	811	689	1066		
3/20/43	703	714	781	1284		
3/22/43	766	879	788	676		
Mean	777	995	933	966	918	

they have a greatly reduced metabolism, and also show pronounced physical symptoms of deficiency that can be corrected only by thyroidal hormone.

For the experiment to be reported, four thyroidectomized goats with long-standing thyroid deficiency were used. These animals were thyroidectomized shortly after birth in the spring of 1941. Shortly after thyroidectomy, they were given sufficient thyroprotein to maintain them in a healthy condition, this treatment being continued until March 21, 1942. At this time the thyroprotein therapy was stopped completely and a hypothyroid condition allowed to develop.

The goats were trained for measurements of oxygen consumption by the spirometer method. During a preliminary period from March 1 to March 5, 1943, measurements of their resting oxygen consumption were made daily. They were then given 5 lbs. of milk daily per goat from 2 Jersey cows that

were receiving 20 grams of thyroprotein daily in their feed. The milk diet was started on March 5 and stopped on March 19. Frequent measurements of metabolism were made during this period. Since thyroid hormone, if present, is known to exert its effect for a considerable time, the determinations were continued to March 22.

The results are given in table I, the data having been converted to the basis of metabolism in calories per day.

During the preliminary period, the average metabolism was 924 calories per day. During 9 days after the experimental milk was fed, the average value was 918 calories per day. Although fairly large day to day variations in metabolism of individual goats were observed, these are no greater than are encountered normally. No definite trend toward a higher metabolic level due to feeding the milk from thyroprotein-fed cows is apparent in the records of any of the animals.

Of at least equal significance is the fact that no evidence of correction of the thyroid deficiency symptoms of the goats was observed during the milk-feeding period. Prior to starting this trial, goats No. 26 and 40 in particular showed extreme symptoms of hypothyroidism. They were very weak, dull-looking, paunchy, and sluggish in their movements and general appearance. The hair was dull and coarse and the skin thick and scaly. No improvement was noted while the milk was fed.

At the close of the experiment each goat was given  $\frac{1}{4}$  gram of synthetic thyroprotein daily for a period of two weeks. Immediate improvement was noted. Within a week the goats became stronger, more alert, less paunchy, and their appetites improved. The coarse dull hair and outer layers of the thick, scaly hide were shed, followed by growth of a new, finer coat of hair.

On the basis of these results, it must be concluded that no evidence of transmission of thyroid hormone into the milk could be detected, either quantitatively by measurement of the metabolism of the milk fed test animals, or qualitatively by improvement of their cretinous condition.

*Experiments with man.* Finally an experiment was set up to determine the influence of milk from "Protamone-fed" cows upon the basal metabolic rate in man. The metabolism was determined by means of a Benedict-Roth Recording metabolism apparatus. The records were taken in the morning immediately upon arising. During the initial period, one quart of normal cow's milk was consumed daily. This period was followed by a period during which milk was consumed from cows fed "Protamone." Following these periods, "Protamone" was taken orally in amounts sufficient to definitely increase the metabolism. Then all thyroidal treatment was discontinued.

Subject I. A girl 17 years old weighing 120 lbs. showed an average B.M.R. of -2.33 per cent during a period of 16 days in which time one quart daily of normal herd milk was consumed. She then switched to milk from

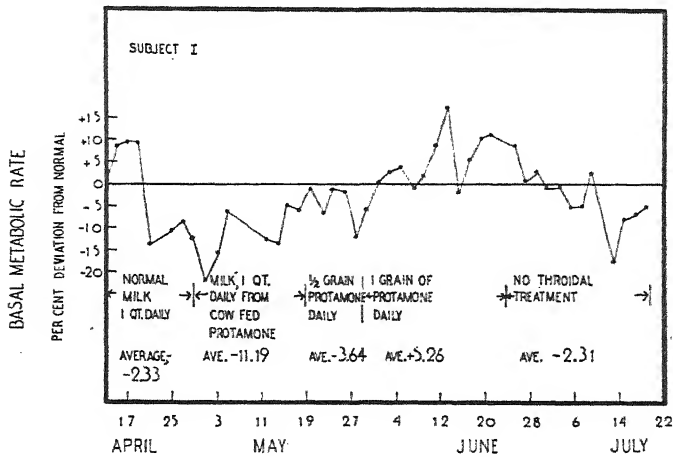


FIG. 3. The effect of milk from "Protamone-fed" cows on the basal metabolic rate in man. During ingestion of 1 quart daily of experimental milk by a 17-year-old girl the basal metabolic rate remained within her usual limits. Administration of "Protamone" directly caused a significant rise in metabolism.

cattle receiving the recommended dosage of "Protamone." Results of the metabolism determinations are given in figure 3. During a period of 20 days on milk from "Protamone-fed" cows her average B.M.R. was -11.19 per cent. For a period of 10 days, she next received daily  $\frac{1}{2}$  grain of "Protamone" orally in a gelatin capsule. As her B.M.R. still continued below normal (ave. -3.64%), the amount of "Protamone" was raised to 1 grain

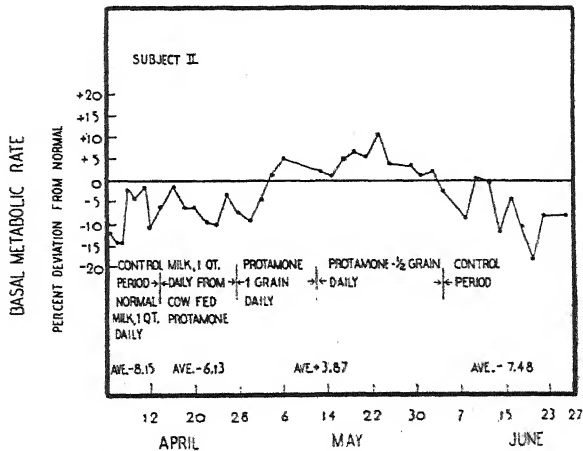


FIG. 4. The effect of milk from "Protamone-fed" cows on the basal metabolic rate in man. One quart of milk from "Protamone-fed" cows taken daily by a 46-year-old man caused no significant deviation from the subject's usual metabolic level. Oral administration of "Protamone" caused a rapid and significant rise in the basal metabolic rate.

daily. On this amount there was an appreciable rise in energy metabolism passing to positive readings in 4 days. The average B.M.R. of the 24-day period was  $-5.26$ . Upon the withdrawal of "Protamone," the metabolism began to decline again and during the month the average B.M.R. was  $-2.31$ . It is clear from this record that the consumption of one quart of milk per day from cattle fed "Protamone" had no effect upon energy metabolism.

Subject II. A man 46 years old weighing 192 lbs. showed a B.M.R. of  $-8.15$  during a period of 10 days during which one quart of milk from normal cows was consumed daily. During the next 2 weeks 1 quart of milk was consumed daily from cows fed "Protamone." The B.M.R. averaged  $-6.13$ , being in the same range as before. However, upon taking 1 grain daily of "Protamone," the metabolism began to increase immediately reaching a first peak in 6 days. The "Protamone" dosage was then reduced to  $\frac{1}{2}$  grain and continued for 23 days during which time the average B.M.R. was  $+3.87$ . Upon stopping "Protamone" the B.M.R. declined rapidly. These results are shown graphically in figure 4. It is obvious that the consumption of one quart of milk daily from cows being fed "Protamone" had no effect on the B.M.R.;  $\frac{1}{2}$  grain of "Protamone," however, was effective in increasing energy metabolism.

#### DISCUSSION

No evidence could be found for the transmission of thyroxine into milk, as determined by biological tests, even though the cows producing the milk were fed considerable amounts of thyroactive material.

Whether or not such transference could be found would depend upon such factors as (a) the permeability of the milk secreting cells to thyroid hormone, (b) the effective level of hormone in the blood stream, and (c) the sensitivity of test methods for detecting the hormone in the milk.

With regard to the first factor, it is known that the mammary gland is readily permeable to simple iodine-containing compounds such as iodides, since feeding of iodide or iodine-rich feeds is reflected almost at once by an increase in the iodine content of the milk. In the case of "Protamone," however, the active iodine is in firm combination in the thyroxine molecule, which in turn is combined as a constituent amino acid of the iodinated casein. Subsequent to digestion and absorption, it is not entirely established in what form the thyroxine molecule would be combined. It is known that thyroxine is stored in the thyroid as a constituent amino acid of thyroglobulin, a giant protein molecule with a molecular weight of approximately 700,000. It is believed that the circulating hormone is similar to thyroglobulin if not identical with it. Even though evidence has been reported by Graham *et al.* (12) and Reineke *et al.* (27) that the mammary gland is permeable to certain fractions of the serum globulins, it appears extremely unlikely that the milk-secreting cells would allow passage of a huge molecule such as thyroglobulin.

The dosage of "Protamone" employed in at least one of these cases was far above what would ever be used in practice for stimulating increased milk and milk fat production. Therefore, the level of hormone in the blood should certainly have been high enough to test its possible transference under practical conditions.

Finally, as to the sensitivity of the tests employed, it has been repeatedly observed that guinea pigs are among the most sensitive of the laboratory animals to thyroidal stimulation. If physiologically significant amounts of thyroidal hormone had appeared in the milk it should have been detected under the conditions of these experiments.

Therefore, it is concluded that the mammary gland does not permit passage of biologically detectable amounts of thyroidal hormone, when "Protamone" is given as a portion of the diet.

#### SUMMARY AND CONCLUSIONS

In view of the recent development of highly active synthetic thyroprotein (Protamone) and the possible use of this product for stimulation of increased lactation in dairy cattle, a series of experiments were conducted to determine whether cows fed "Protamone" would transmit any thyroidal substance in their milk.

In one experiment guinea pigs were given approximately 100 ml. of milk daily for two weeks from cows receiving massive doses of "Protamone." In a second trial with guinea pigs, milk from cows receiving a moderate dose of "Protamone" was fed for six weeks. Frequent determinations of metabolism during both experiments failed to show any significant differences between these animals and paired controls receiving a normal milk diet, or normal guinea pigs on a stock diet. Responsiveness of the guinea pigs to thyroidal stimulation was shown by the fact that the experimental group while still on the milk diet showed a 25 per cent increase in metabolism when 2.5 micrograms of l-thyroxine was injected daily for two weeks.

In a similar experiment with thyroidectomized goats no metabolic stimulation or subjective improvement of the cretinism could be detected as a result of feeding milk from "Protamone-fed" cows for two weeks. These animals responded markedly, however, when  $\frac{1}{4}$  gram of "Protamone" daily was fed directly.

No elevation of the basal metabolism could be detected when a quart of milk daily from "Protamone-fed" cows was consumed by two human subjects. Significant elevation of the basal metabolic rate occurred when  $\frac{1}{2}$  to 1 grain of "Protamone" daily was given orally.

On the basis of these experiments it was concluded that the mammary gland does not permit passage of biologically detectable amounts of thyroidal hormone when "Protamone" is fed to lactating cows.

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## THE EFFECT OF THE METHOD OF MANUFACTURE OF BUTTEROIL ON ITS KEEPING QUALITY\*

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For centuries butteroil has been prepared and used in Egypt, India, and other countries in the Middle East, where it is called Samn, Ghee, and Maslee respectively. The preparation is usually made in small units, and the process is called "boiling-off." Its origin is not well known, since it is a development of traditional farmhouse practice. The method consists mainly of heating the butter in an open kettle with continuous stirring until all the water is boiled off. The non-fat solids precipitate to form a brown sediment, which can be strained from the butteroil. This sediment (called Mourta in Egypt, Chhas in India, and Ankenrume or Lure in Switzerland and Germany) is used as a spread, in cooking, or is fed to animals. Although the boiling-off method is being used in Switzerland, Sweden, France, and other European countries, there is no definite standard procedure with respect to temperature control. Butteroil, when correctly prepared by this method, acquires a special pleasant aroma.

Parnerker (24) has recently recommended to Indian farmers a modified method designed for saving both fuel and fat. In this process the butter is melted and allowed to stand in a cool place to solidify the fat; then the butter serum is drained off, and the fat is boiled in the usual manner.

The modern ways of preparing butteroil on a commercial scale may well be called the centrifugal methods. These are used in New Zealand, Australia, and Germany, and have recently been recommended in the United States (12). In all these methods the butter serum is partly removed by the centrifuge; but the details differ in each country. The butteroil thus prepared has a pleasant, delicate flavor, but lacks the aroma of that obtained by boiling-off process.

New Zealand has developed a continuous process by which low-moisture, unsalted butter and unsalted whey butter are converted into butteroil (28). The butter is melted by direct steam; the oil is floated off; the oil in the serum

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is recovered by centrifuging and is combined with the main bulk of the oil. The oil is then passed in turn through the separator, heater, separator, dehydrator, and rotary cooler, and finally into specially constructed containers.

In the Australian method (1) the butter serum is adjusted to pH 10 by the addition of caustic soda. This alkalization is claimed to be beneficial in increasing the rate of separation and the speed of dehydration. The mixed melted butter is stirred, passed through two whey separators, without gravity separation or additional heating, and fed directly into a continuous vacreator. It is cooled in an internal tubular cooler and packed. The Australian and New Zealand butteroil thus prepared is called dry butterfat.

In the German method suggested by Mohr (20) in 1940, the butter is melted, clarified, and centrifuged at 70° C.; then the fat is heated at 105–115° C., cooled, and packed.

The recent method of Holm (12, 13), which is suggested for use in the United States, does not appear to have been applied to large scale manufacturing. The object, apparently, is to produce butteroil in highly purified form; and the method depends upon removal of oxygen and packaging of the product in an inert gas for preservation against oxidative deterioration. The butter is melted, the serum is separated by gravity, and the fat is passed through a cream separator to remove the excess water and curd. If the fat is not entirely clear, it is washed with warm water and re-separated. It is then vacuum dried with agitation to remove the final traces of water and gases. The oil is vacuum packed and sealed under an atmosphere of nitrogen (14).

The conventional procedure for preparing butteroil in the research laboratory may be called the filtration method. Butter melted at about 50° C. is left to separate into three layers. The serum layer at the bottom is drawn off, the yellowish white layer at the top is skimmed away, and the middle butteroil layer is filtered. The crystal-clear product thus prepared is used for studies related to milk fat.

✓ Butteroil of commercial purity should contain not more than 0.1 per cent of either water or non-fat solids. The water content is of special significance, since it may vary between 0.08 and 0.3 per cent. As shown earlier, (31, V) a water content above 0.15 per cent may cause rusting of the metal containers unless the butteroil is forced to form fine crystals by rapid cooling or inoculation. If the moisture is 3 per cent or over, the oil may become susceptible to spoilage by microorganisms, a condition that rarely exists where proper control is maintained.

The main type of deterioration which develops in butteroil on prolonged storage is that of oxidative rancidity, and one may naturally suspect that resistance to oxidation may vary with the method of preparation. The war emergency has emphasized this problem, since the shipping of butteroil to the armed forces and to the allied and dependent nations in place of butter

would save 20 per cent tonnage and eliminate or greatly reduce the need of refrigeration in transportation and storage. Another problem concerns the effect of the manufacturing method on the preservation of the vitamin A and carotene content, both in processing and in storage.

Patil and Hammer (25) compared the stability of filtered butteroil not heated above 55° C. with that of ghee prepared by boiling-off and brought to 140° C. They concluded that a high temperature of heating is not necessary, since the keeping quality of the two during 23 weeks of storage at room temperature was about the same. Ritter (29), on the other hand, after a similar comparison, concluded that butteroil prepared by the boiling-off method is more resistant to the development of oxidized flavor than filtered fat. Mohr (19), comparing butteroils filtered at 50° and 90° C. and ghee prepared at 110° C. found that after 7 months the ghee was superior, but that the difference had disappeared by the end of 11 months. According to Ritter and Nussbaumer (30), butteroil is less susceptible to oxidation when filtered at 100° rather than 42° C. Later Ewbank and Gould (6) concluded that heating butter or butteroil to 127° C. for 30 minutes hastens the oxidation. The heated butter was filtered at 80° to 90° C., but, as they pointed out, when a similar sample is filtered at 45° C. the resulting butteroil is more stable than heated butteroil.

The effect of processing on the vitamin A activity of butteroil has been studied somewhat. Grewal (9) reported that butteroil prepared by the centrifugal method is higher than that by the Indian method. According to Banerjee and Dastur (2), vitamin A in ghee is fairly stable up to 125° C. being rapidly destroyed at higher temperatures; and the vitamin in the oil prepared from cow's milk is more stable to heat than in that from buffalo's milk. They attributed the difference to the relative absence of carotene in the buffalo milk fat. Muthanad and Seshan (21) later found that ghee heated to 100° C. for 5 minutes lost some vitamin A.

#### PREPARATION OF BUTTEROIL

Sweet cream was churned in glass, and the resulting butter was washed and used for preparing butteroil by the following methods:

1. The butter was melted at 70° C. in specially constructed glass equipment enclosed in a black-painted wooden case to minimize the effect of light. The equipment consists of a glass tube  $3\frac{1}{4}$  inches in diameter and about 11 inches long, connected at the upper flanged end with a flanged, removable dome that has ground-glass connections to a vacuum line, a stirring motor, and a thermometer. The lower end of the tube is tapered and connected to a two-way vacuum stopcock. The tube is surrounded with a water jacket, through which water is circulated from a thermostatically controlled water bath. After the butter was melted, the butter serum was drawn from the bottom through one of the stopcocks, the other being left for drawing the

butteroil. The milk fat was then dried with continuous stirring at 70° C. under 26 inches of vacuum. The dry butteroil, which was partly cloudy, was clarified by centrifuging in bottles for 5 minutes at a low speed. The process took about 90 minutes and was planned to be comparable in the essential steps to some centrifuged methods.

2. The butter was treated exactly like the previous sample except that after it was melted and the butter serum was separated by gravity and drawn off, the fat was washed twice with distilled water at 70° C., then dried under vacuum and centrifuged in bottles. This type was prepared to show how the purifying of butteroil by washing affects its keeping quality.

3. Butteroil was prepared by heating the butter with continuous stirring in a glass beaker over a Bunsen burner to 105°–110° C. until all the water had been evaporated and the non-fat solids settled. The temperature was then raised to 130° C. As the temperature approached 120° C., the butteroil formed a relatively stable foam, which dissolved back in the fat on cooling. The fat was dark and had the characteristic aroma of the butteroil prepared by the boiling-off process with an additional heated flavor and odor. The fat was then clarified by centrifuging in the same way as the samples 1 and 2 (above), and the dark brown sediment removed. The process took about 75 minutes.

4. The butteroil was prepared by the boiling-off method, but its temperature was not permitted to rise above 110° C. It was then centrifuged in bottles. The resulting fat was clear, appeared slightly darker than the washed fat (sample 2), and had a special pleasant aroma, with no heated odor. The process took about 60 minutes.

5. The butter was melted in an oven at 70° C., the top layer was skimmed away and the serum drawn off, and the fat was filtered in the usual manner. The resulting fat was crystal clear. The process took about 150 minutes—the time obviously depending upon the volume of fat and the number of filters used.

6. Butteroil prepared by the filtration method (sample 5) was heated to 130° C. for 30 minutes. Significantly, this fat did not foam even up to a temperature of 145° C.

In each set of experiments, butter from the same lot was used for preparing samples described above. Then the butteroil, divided into small portions, was stored at 2° C.

#### THE EFFECT OF THE METHOD OF PREPARATION ON THE RESISTANCE OF BUTTEROIL TO OXIDATION

The method used to determine the peroxide values of the butteroil is Wheeler's as modified by Henderson and Young (11). From each sample 4-ml. portions were heated in a thermostatically controlled oil bath at 79.5° C. shielded from direct sunlight. The values reported are millimoles

of peroxide per 1000 grams of fat. The results of different sets of experiments were quite uniform and are represented in figure 1.

As this figure shows, the method of preparation markedly affects the stability of the resulting butteroil. In this experiment the butter was prepared from raw cream of high quality. Evidently the boiling-off method gives the most stable fat; and the stability is slightly greater in sample 3, heated to 130° C. than in sample 4, heated to 110° C. Clearly, too, the filtered fat is less stable than samples 1 and 2, which were dried under vacuum and then clarified by centrifuging. Unlike the case of sample 3.

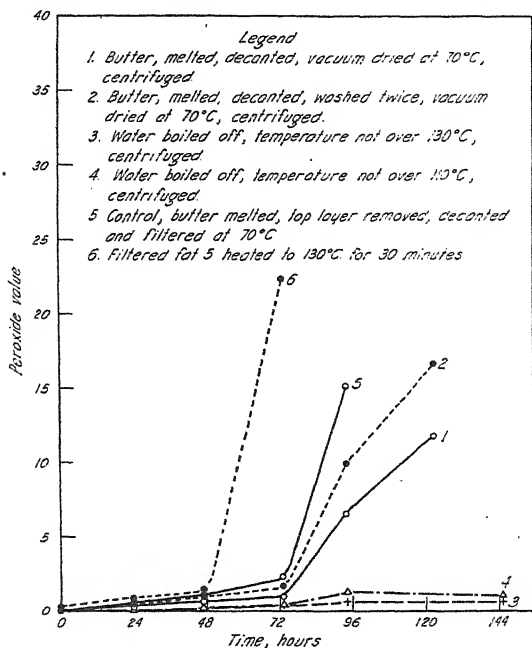


Fig 1. The effect of the method of preparation on the resistance of butteroil to oxidation at 79.5°C (Made from raw cream butter)

heating the filtered fat to 130° C. (sample 6) impaired its stability. Sometimes, in comparing samples similar to 1 and 2, where the only difference in treatment is the washing given No. 2, the difference in stability was even greater than is shown in figure 1. As before, washing the butteroil decreased its resistance to oxidation. In one set of experiments, for example, the washed fat equivalent to sample 2 had passed the end of its induction period after 120 hours (which is taken arbitrarily as a peroxide value of 5), while the untreated sample 1 had reached a value of only 2.1.

#### THE ACID VALUES OF BUTTEROIL

High acidity of butteroil was reported to have an undesirable effect on the stability of fat against oxidation and the destruction of vitamin A by

heat (3). The acid values of butteroils were determined by the Official Method and reported as per cent oleic in table 1. These values are approximately the same except for sample 3, where butteroil was prepared by the boiling-off method at 130° C. The difference, however, is not significant, since it was not evident when the experiments were repeated. In another trial when the butteroil was prepared from pasteurized cream butter, the acid value was found to be low, ranging between 0.12 and 0.16 per cent. Apparently, therefore, the method of preparation has no significant effect on the hydrolysis of the fat.

TABLE 1

*The effect of the method of preparation of butteroil on its acid values, phosphorus content and vitamin A activity (butteroil from raw-cream-butter)*

Method of preparation	Acid value as oleic	Phosphorus as lecithin	Carotene	Vitamin A	Total reducing substances as tocopherol
	%	%	γ/gm.	γ/gm.	γ/gm.
1. Butter melted, decanted, vacuum dried at 70° C., centrifuged .....	0.365	0.151	3.39	4.89	146
2. Butter melted, decanted, washed twice, vacuum dried at 70° C., centrifuged .....	0.326	0.0114	3.52	4.81	137
3. Water boiled off at 105-110° C., temperature increased to 130° C., centrifuged .....	0.444	0.0805	3.23	4.32	364
4. Water boiled off, temperature not over 110° C., centrifuged .....	0.385	0.0503	3.27	4.27	214
5. Control, butter melted, top layer removed, decanted and filtered at 70° C.	0.365	0.0073	3.58	4.61	130
6. Number five fat heated to 130° C. for 30 minutes	0.370	.....	3.43	4.57	112

THE EFFECT OF THE METHOD OF PREPARATION ON THE VITAMIN A  
AND CAROTENE CONTENT OF THE BUTTEROIL<sup>2</sup>

The Carr-Price antimony trichloride reaction was used for vitamin A determination, the readings being taken at the wave length of 620 mμ, using a Coleman Universal Spectrophotometer, Model 11. The carotene content was determined colorimetrically by extracting the color from the ether-soluble, unsaponifiable fraction of the butteroil, using Skellysolve B, extracting the xanthophyll with methanol, and reading at the wave length of 440 mμ. The values are expressed as micrograms of vitamin A and Beta carotene per gram of butteroil.

<sup>2</sup> The authors are indebted to Miss M. L. Long for these determinations.



Judging from table 1, the method of preparation has little or no effect upon the preservation of the vitamin A activity during processing. The differences are not considered significant inasmuch as they are not consistent from one set of samples to another.

#### TOTAL REDUCING SUBSTANCES MEASURED AS TOCOPHEROL

The tocopherols, being known to act as antioxidants for fats (22), might account for the differences in the stability of the butteroil. Vitamin A and carotene are known to interfere with the determination; but, since all samples contained essentially the same concentration of vitamin A and carotene, analyses were made directly on the fat. Into a 10-ml. volumetric flask were weighed 4 grams of the butteroil; and the volume was made up with hexane. To 5 ml. of the fat solution was added 20 ml. of Emmerie and Engel reagent (5). The colorimetric readings were made with a Coleman spectrophotometer Model 11 at 525  $m\mu$ , after 45 minutes, using a solution that contains the reagents and 1.4 grams pure triolein as a reference. The readings were converted to tocopherol values from a calibration curve (made with  $\alpha$ -tocopherol) and are reported in table 1 as the total reducing substances.

These results show, for the reducing substances of butteroils, a wide variation that cannot reasonably be attributed to variation in the tocopherol content. Significantly, however, these total reducing substances can be directly correlated with the stability of the fat against oxidation. They are highest in the butteroils prepared by the boiling-off method, lowest in the filtered fat that is heated to 130° C.

As further work has shown, the compounds that have the sulfhydryl group, such as glutathione, give a red color with the Emmerie-Engel reagent. Further experiments were planned, therefore, to determine how glutathione affects the oxidation of butteroil.

In this experiment filtered fat was used and divided into two portions. To the first lot 50 mg. of pure glutathione dissolved into 10 ml. of water was added to 100 ml. of fat; the water was then boiled off by heating in an oven at 100° C. and 20 inches vacuum. The other lot was used as a control; 10 ml. of distilled water per 100 ml. of fat was added to it, and the water boiled off in the same manner. The fat containing the glutathione reached the end of its induction period in 96 hours, as compared with 24 hours for the control.

We may reasonably assume, therefore, that sulfhydryl groups produced by the effect of heat on the protein part of the fat globule membrane (8), and probably other reducing substances are transferred to the butteroil in the process of its preparation by the boiling-off method and there act as antioxidants. This assumption may explain part of the high reducing substances in samples 3 and 4 and may be related to their high stability. No specific method for determining the sulfhydryl groups in butteroil was available; but these groups do evidence themselves by the heated odor and flavor in sample 3.

THE EFFECT OF  $\alpha$ -TOCOPHEROL ON THE STABILITY OF BUTTEROIL

The previous experiment did not exclude the possibility that variation in the tocopherol content of the butteroil is a factor in the resistance to oxidation. Pure  $\alpha$ -tocopherol was added, therefore, to the filtered fat; the effect on stability was observed. Figure 2 shows the amounts added and the results obtained.

Judging from the peroxide-accumulation curves, the activity of  $\alpha$ -tocopherol as an antioxidant is not proportionate to its concentration. If a per-

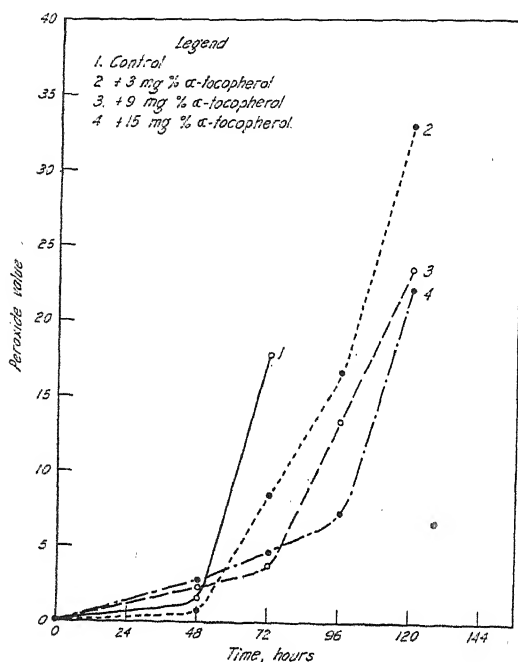


Fig. 2 The effect of adding  $\alpha$ -tocopherol to butteroil on its resistance to oxidation at 79.5 °C.

oxide value of 5 is assumed to be the end of the induction period, then both samples that contain 9 mg. and 15 mg. per cent  $\alpha$ -tocopherol would have about the same induction period. The accumulation of the peroxides during the induction period is less for the sample to which 3 mg. per cent  $\alpha$ -tocopherol is added. These findings agree with the report of Swift, Rose, and Jamieson (32) on the effect of adding  $\alpha$ -tocopherol to the methyl esters of cottonseed oil. The addition of as much as 15 mg. per cent  $\alpha$ -tocopherol does not make the filtered fat nearly so stable as that prepared by the boiling-off method. Probably, then, the additional stability of samples 3 and 4 does not result from a more favorable transfer of tocopherol to the fat by the method of preparation.

THE EFFECT OF THE METHOD OF PREPARATION ON THE PHOSPHORUS  
CONTENT OF THE BUTTEROIL

The phospholipids occur naturally in milk and are highly concentrated around its fat globules as phospholipid-protein complexes. These compounds are known to act as antioxidants in fats, and several individual fractions have been patented. The literature on this subject has been reviewed recently by Mitchell and Black (18). It is also common knowledge that the phospholipids are surface active and tend to orient themselves at the interfaces. If, therefore, the water phase is completely removed by boiling, where do the phospholipids go? To test this point the butteroils were ashed according to Halliday (10), and the phosphorus was determined by the colorimetric method of Fiske and Subbarow (7). The results were converted to per cent lecithin by assuming that the phosphorus in the sample was combined in a stearyloley l lecithin containing 3.85 per cent phosphorus.

As table 1 indicates, in preparing the butteroil by the boiling-off method (sample 3) the phosphorus content of the fat calculated as per cent lecithin increases from 0.0073 in the filtered fat to 0.0805 in the heated fat. The phosphorus content of the butteroils is directly correlated with its stability against oxidation. Apparently, therefore, the most satisfactory method of preparation is the one that ensures the transfer of a high phospholipid value to the fat. Part of this phosphorus might originate in a protein fraction held as a suspenoid in the fat. Additional experiments were planned to ascertain how the addition of phospholipid to the filtered butteroil will affect the stability against oxidation.

## THE EFFECT OF ADDING PHOSPHOLIPID ON THE STABILITY OF BUTTEROIL

Commercial soybean phospholipid was used as a source, from which the partly purified phospholipid was obtained by repeated extraction with ether and precipitation from acetone. The cream-white phospholipid thus secured was added to the filtered fat in concentrations that approximate those found in the different butteroils prepared. Figure 3 reports the results.

The peroxide-accumulation curve shows the striking effect of increasing concentrations of phospholipid on the stability of the butteroil. The sample that had 0.1 per cent phospholipid approached but did not quite equal the stability of the butteroils prepared by the boiling-off methods. Conceivably, the phospholipid in this concentration might increase the stability of the fat if it acted synergistically with  $\alpha$ -tocopherol.

THE SYNERGISM BETWEEN PHOSPHOLIPID AND  $\alpha$ -TOCOPHEROL IN BUTTEROIL

One of the phospholipids, cephalin, has been shown by Olecott and Mattill (23) to co-act with the tocopherols in a most remarkable manner, in stabilizing the purified fatty acids esters and lard against oxidation. To test this

co-action or synergism in butteroil,  $\alpha$ -tocopherol and purified soybean phospholipid were added to filtered fat in three different combinations. For the sake of comparison the same fat was used in samples reported in figures 2, 3, and 4.

The peroxide curves in figure 4 show some interesting facts. There is some synergetic action on adding small amounts of phospholipid and  $\alpha$ -tocopherol. (Compare No. 2 fat—figures 2, 3, and 4.) On the other hand, when the amount of phospholipid added is 0.10 per cent with various amounts of tocopherol, the protective action seems to be due to the presence

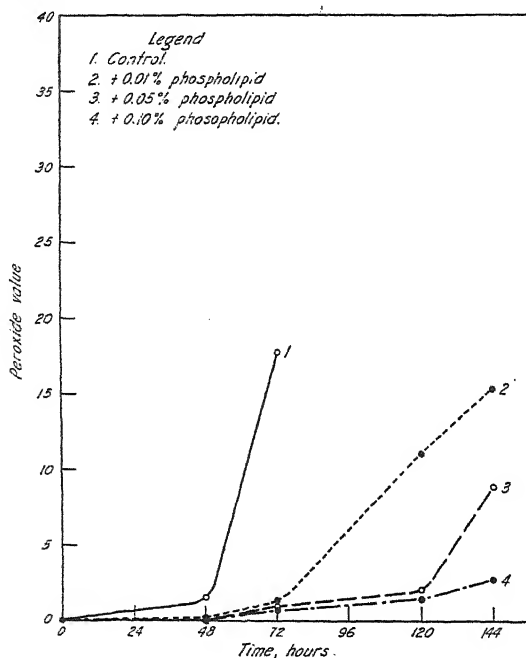


Fig. 3 The effect of adding purified soybean phospholipid to butteroil on its resistance to oxidation at 79.5°C

of phospholipid, and the addition of 15 mg. per cent  $\alpha$ -tocopherol seems to be less beneficial than adding 3 mg. per cent. (Compare fats 3 and 4 in figure 4 with No. 4 in figure 3.)

These results show, however, that the synergetic action of the phospholipid and  $\alpha$ -tocopherol does not account completely for the stability of the butteroil prepared by boiling.

#### DISCUSSION

Most important of the deteriorative changes that may occur in fats and oils on storage is oxidative rancidity; and butteroil is no exception. A recent review on the subject (18) summarizes the logical means of preventing these reactions as being either protection from oxygen, as exemplified

by vacuum packing, or the use of antioxidants. Evidence is here presented to show that the method of preparing butteroil from butter has a marked influence on its resistance to oxidation. Assuming that the end of the induction period is reached when the peroxide value is 5, the data indicate that butteroil is least stable when prepared by filtration. Washing of the fat is also detrimental to its stability. On the other hand, butteroil prepared by boiling off is shown to be considerably more stable; but it acquires a distinct heated odor and flavor if the temperature of the fat rises above

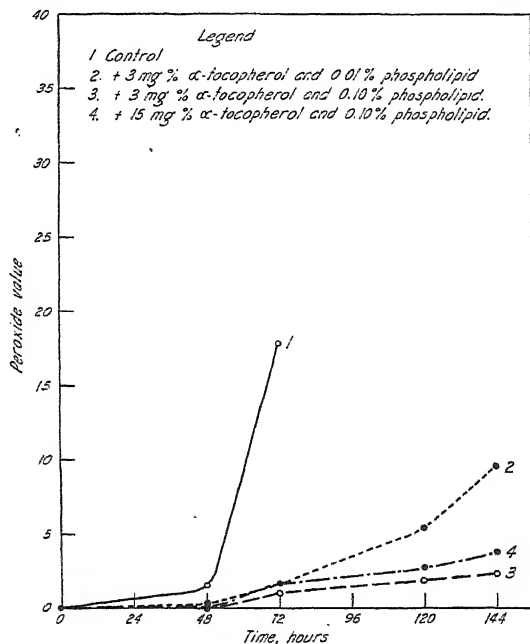


Fig. 4 The effect of adding  $\alpha$ -tocopherol and purified soybean phospholipid to butteroil on its resistance to oxidation at 70.5°C.

110° C. The additional stability gained by heating to 130° C. in the boiling-off method is not believed to be great enough to offset the disadvantage of a cooked flavor.

The stability of butteroil is found to be associated with an increase in the phosphorus content and the total reducing substances in the fat. The vitamin A, the carotene, and the acid values of the fat do not change appreciably with the manipulations used. The stability of butteroil made by the boiling-off method cannot well be attributed to a destruction of certain oxidative enzymes (20), because heating filtered fat to 130° C. reduces its stability. Likewise, the shortening of the induction period of the latter fat may not be due to the destruction of natural antioxidants, as others have suggested

(6, 30, I), but more likely is caused by pushing the fat further towards the end of its induction period during the heating. According to Ritter and Nussbaumer (31, VI), diacetyl acts as a prooxidant in butteroil; conceivably, therefore, when these are removed by steam distillation in the boiling-off process the stability of the fat increases. The results of experiments not reported herein show, however, that when 10 to 20 per cent water is added to the filtered butteroil and then removed by boiling, the stability of the fat is reduced.

The data reported support the assumption that when butteroil is prepared from butter by boiling off the water in the presence of the non-oil phase, the protein of the phospholipid-protein complexes in the non-oil residue is so denatured that the phospholipids are free to dissolve in the oil. An increase in the stability and foaming capacity of the oil results. This assumption, previously made (27, 29), is substantiated by the fact that good stability is associated with a high phosphorus content. It is also strengthened by the data (fig. 4) showing that the addition of purified soybean phospholipid increases the stability of filtered butteroil. The significance of the freeing of an organic compound from its protein posthetic group by means of heat is being increasingly recognized in relation to vitamin preservation during dehydration or storage of foods (4, 26).

Judging from comparison of sample 4 in figure 2 with sample 3 in figure 4, however, the phospholipid content is not the only factor that accounts for the differences in their stability, since both fats contain about 0.05 per cent phospholipid. The higher content of the total reducing substances in the former sample probably also contributes to its stability. These compounds reduce ferric chloride to ferrous, as is shown by their reaction with the Emmerie-Engel reagent. They cannot, however, all be considered as tocopherol, since the sulfhydryl group present in glutathione interferes with the test; this group also prolongs the induction period of the butteroil. These groups are known to be released from the proteins surrounding the fat globules in milk by the effect of heat; they act as antioxidants (8, 15). The liberation of the heat-volatile sulfides of milk is of great current interest (33). Though there seems to be no chemical test to determine these groups in fats, they evidence themselves by the cooked flavor they impart to fats heated to 130° C. in the presence of butter serum. Other reducing groups produced by the heating of the proteins belong to tyrosine and tryptophane (17). Marvel (16) patented the use of tyrosine and its esters as antioxidants in edible fats and oils. Attempts to determine these specific groups in butteroil prepared by the boiling-off method are in progress.

The addition of  $\alpha$ -tocopherol to the filtered fat does not prolong the induction period of the latter to the same extent as in the boiled-down fat. In the presence of 0.1 per cent phospholipid, the addition of 3 mg. per cent of  $\alpha$ -tocopherol seems to be more beneficial than larger amounts, and some

synergism is observed. Unless the tocopherols in milk are shown to be largely associated with the proteins in a complex form, one cannot well conceive that a wide variation occurs in the tocopherol content of butteroils prepared by different methods. The values reported in table 1 are calculated as tocopherols for convenience only. They indicate, however, the concentration of all the substances present in the butteroil that reduce ferric chloride in 45 minutes. All these seem to act synergistically with the phospholipids in protecting the boiled-down butteroil against oxidative rancidity.

No method of manufacture tested appreciably affects the vitamin A and the carotene content of the fat. The higher concentration of compounds of known biological value—namely, the phospholipids—in the oils prepared by certain methods may add to nutritional value, as well as to stability. Increased stability indicates increased protection against the destruction of vitamin A activity.

#### SUMMARY

Butteroil\* made by the process in which butter is heated to 110° C. to drive off the moisture, the residue being removed by centrifuging, has been found to be more resistant to oxidative rancidity than when lower temperatures of isolation of the oil are used. The improved keeping quality has been shown to result from the transfer of greater amounts of phospholipid material from the non-oil phase of butter to the oil, presumably because of the denaturing action of heat on the protein of the phospholipid-protein complex. The concentration of reducing substances is also higher in the butteroil made by the "boiling-off" process than by the low heat treatment.

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\* The authors feel that 'dry milk fat' would be a more descriptive terminology, and essentially correct.

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## THE NUTRITIVE VALUE OF ALFALFA HAY. II. STARCH AND GLUCOSE AS SUPPLEMENTS TO AN ALL ALFALFA HAY RATION\*

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It has been recognized for a long time that when a hay crop is fed alone it does not produce growth, meat or milk as efficiently, per pound of ingested total digestible nutrients, as when the ration is supplemented with concentrates. Several investigators have reported that a ration composed entirely of alfalfa hay appeared to be deficient in some factor or factors necessary for milk production (37, 33, 15, 14, 22, 17). Graves *et al.* (13), however, found that the average yearly production of a group of cows was 376 pounds of butterfat on a ration of alfalfa hay alone, which indicated efficient utilization of the total digestible nutrients. These investigators reported also that another group of cows produced 395 and 357 pounds of butterfat per year on alfalfa alone during their first and second lactation periods. Dawson and co-workers (5) found that a group of cows produced 392 pounds of butterfat on alfalfa cut at the initial-bloom stage, 326 pounds on alfalfa cut at the half-bloom stage, and 311 pounds when the alfalfa was cut at full-bloom. According to the results obtained by Willard (36) on an all hay ration, cows increased their milk production when they were changed from poor quality alfalfa to a better quality alfalfa hay. It appears from a review of the literature that occasionally certain alfalfa hays are more efficient than others for milk production when fed alone.

The factor or factors in grain which increase the nutritive value of alfalfa hay may be available energy. Crampton (4) fed one group of rabbits mixed pasture grasses alone, a second group received mixed pasture grasses plus 5 per cent casein and a third group received mixed pasture grasses plus 5 per cent sucrose. The group on grass alone lost 134 grams in weight whereas the groups that received the casein and sucrose supplements each gained 30 grams in weight. It was concluded that the failure of mixed grasses to produce gains in weight was due to a lack of available energy. Kellner and Köhler (24) studied the effect of adding pure nutrients to a super maintenance ration of steers. They concluded that the feeding of one kilogram of digestible protein, starch, cane sugar, crude fiber or fat per day resulted in the production of 235 gm., 248 gm., 188 gm., 253 gm. and 474-598 gm. of fat, respectively. Similar experiments with concentrates and roughages showed

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that the digestible nutrients in roughages produced less than the calculated amount of fat.

Turk and associates (35) observed that the biological value of the protein in alfalfa hay was 50 when the hay was fed to lambs whereas the biological value increased to 72 when the hay was supplemented with starch and sugar. Some investigators have reported that the addition of starch or glucose to the ration of ruminants depressed the utilization of some of the nutrients. Robinson (34) found that the addition of 300 grams of starch had no effect on the digestibility of the crude fiber in grass hay or cereal straw but that the addition of 2,270 grams produced a marked depression. The addition of 300 grams of casein to the ration counteracted the effect of the high starch intake. Hart and Humphrey (16) reported that cows receiving a ration of alfalfa hay and corn starch decline in milk production but that production increased when the ration was changed to corn, corn gluten feed and corn stover. Forbes and associates (9) found that the metabolizable energy of a ration containing alfalfa hay and corn was better utilized by a steer than that of a ration of alfalfa hay alone.

Armsby and Fries (1) stated that corn starch has a gross energy value of 4,105 Calories per kilogram of dry matter and Kellner and Köhler (24) reported the value as 4,176 Calories. These investigators also concluded that the addition of starch to the ration of steers decreased the digestibility. The results obtained by Mitchell and associates (29) with calves indicate that the addition of glucose to the ration did not increase methane production although there was a distinct depression in the digestibility of the crude fiber. They reported that the gross energy value of glucose was 3,680 Calories per kilogram of dry matter.

In a preliminary report (17) it was pointed out that when corn, oats, corn gluten meal or barley replaced an isocaloric amount of alfalfa hay in an all alfalfa hay ration of dairy cows, milk production increased markedly. These data were interpreted as indicating a deficiency in alfalfa hay which was corrected by feeding cereal grains or some of their by-products. A long-time study of supplementing an all alfalfa hay ration of cows with corn, oats or barley by the reversal lactation method is under investigation. The object of this paper is to present the results obtained by supplementing the all alfalfa hay ration with corn starch or corn sugar and then replacing these supplements with corn.

#### EXPERIMENTAL

The cows used in this investigation were placed on an all alfalfa hay ration at the time of calving and remained on this ration until the reserve factor or factors necessary to complete the alfalfa hay for milk production were exhausted. This was indicated by a sharp drop in milk production which usually occurred within 6 to 10 weeks following calving. When corn starch

or corn sugar was added to the ration, the amount of alfalfa was not reduced unless the appetite became affected. The supplement was placed on the hay and the cows consumed it with relish. It was thought that if the basal ration were deficient in a dietary factor other than available energy then milk pro-

TABLE 1  
*Gross energy values of corn starch and glucose as reported in the literature*

Authority	Heat of combustion			T.D.N.
	Cal./kg.	Cal./lb.*	Cal./100 lb.	per 100 lb.†
Corn starch				
Atwater (2) .....	4,094	1,820	182,000	100.3
Stohmann (2, 12) .....	4,123	1,870	187,018	103.1
Stohmann & Langbein (2, 12) ..	4,183	1,897	189,740	104.6
Berthelot & Vieille (2, 12) .....	4,228	1,918	191,781	105.7
Gibson (2) .....	4,164	1,889	188,878	104.1
Stohmann (12) .....	4,116	1,867	186,701	102.9
Rechenberg (12) .....	4,479	2,032	203,166	112.0
Fingerling <i>et al.</i> (8) .....	4,061	1,842	184,206	101.5
Loewy (26) .....	4,182	1,897	189,694	104.6
Stohmann & Langbein (25, 1) ..	4,179	1,896	189,558	104.5
Armsby & Fries (1) .....	4,105	1,862	186,202	102.6
Kellner & Köhler (24) .....	4,176	1,894	189,422	104.4
DuBois (6) .....	4,200	1,905	190,511	105.0
Nakamura (31) .....	3,963	1,798	179,761	99.1
Maynard (27) .....	4,230	1,919	191,872	105.8
Fraps <i>et al.</i> (11) .....	4,089	1,855	185,476	102.2
Mean .....	4,161			103.9
12% moisture .....				91.4
T.D.N. on basis of 93% digestibility (apparent) .....				85.0
Glucose				
Berthelot & Vieille (2, 12) .....	3,762	1,706	170,643	94.1
Stohmann & Langbein (2, 12) ..	3,742	1,697	169,736	93.6
Stohmann (2, 12) .....	3,692	1,675	167,468	92.3
Gibson (2) .....	3,754	1,703	170,280	93.9
Rechenberg (12) .....	3,939	1,787	178,672	98.5
Emery & Benedict (7) .....	3,739	1,696	169,600	93.5
DuBois (6) .....	3,740	1,696	169,645	93.5
Kharasch (25) .....	3,737	1,695	169,509	93.4
Maynard (27) .....	3,760	1,706	170,552	94.0
Karrer & Fioroni (23) .....	3,743	1,698	169,781	93.6
Huffman & Fox (19) .....	3,720	1,687	168,738	93.0
Mitchell <i>et al.</i> (29) .....	3,680	1,669	166,924	92.0
Mean .....	3,751			93.8
7% moisture .....				87.2
T.D.N. on basis of 93% digestibility (apparent) .....				81.1

\* 2.2046 pounds per kilogram used for conversion.

† 1,814 Calories per pound of T.D.N. (3).

duction would not be materially altered. The supplementary feeding period usually lasted 15 days and this test period was checked against the preceding 15-day lactation period. Fifteen-day periods were used, rather than longer periods, in order to minimize the natural decline in milk production.

In calculating the total digestible nutrient intake, the actual coefficient of digestibility obtained by a digestion trial was used for each hay fed. The requirement figures were calculated according to the standard recommended by Morrison (30). The digestible protein values were also calculated. They are not included with these data but a liberal supply was fed at all times.

Commercial grades of corn starch and corn sugar (glucose) were used. The total digestible nutrient values for these supplements were calculated on the basis of the coefficients of digestion of the dry matter of corn starch reported by Armsby and Fries (1). The same digestibility for the dry matter of corn sugar was assumed. The heat of combustion values reported in the literature for starch and glucose were used as a basis for our calculations. The total digestible nutrient value for starch was calculated on the basis of 12 per cent moisture and on the basis of 7 per cent moisture for glucose. Table 1 presents the gross energy values of these supplements calculated from heat of combustion data recorded in the literature.

The care of the cows and the method used to collect representative milk samples have been reported (18).

#### RESULTS

The data obtained from 25 experimental feeding periods on the 14 cows used in this study are presented in tables 2-4. The effects obtained on milk production by supplementing the all alfalfa hay ration with 6 pounds of starch or with 6 pounds of glucose per day are illustrated in table 2. The addition of starch to the ration resulted in a decline in 4 per cent fat-corrected milk in 2 of the 4 trials. Fat production and the test followed the fat-corrected milk. Hay consumption was reduced slightly after the starch was added to the ration (average—1.7 pounds per day). The feeding of 6 pounds of glucose in place of starch gave similar results. Fat-corrected milk declined in 3 of the 6 trials. Glucose decreased the consumption of hay more than the starch (average—5.1 pounds). These results are inconclusive insofar as showing a definite trend in the amount of fat-corrected milk and fat produced but they do illustrate the fact that no marked increase in milk production was obtained by supplementing the alfalfa hay ration with either starch or glucose.

The results obtained by supplementing the alfalfa hay ration with various amounts of starch or glucose followed by increased amounts of starch or glucose or both are given in table 3. The addition of these supplements to the ration resulted in a decrease in 4 per cent fat-corrected milk in all of the trials. When these supplements were increased further, milk production decreased below the previous period in 5 trials and remained unchanged in the sixth. The average yield of fat-corrected milk was 4.2 pounds per day less than that obtained on alfalfa alone. The pounds of fat produced and the test followed the fat-corrected milk. These results show a definite down-

TABLE 2

*Effects obtained in milk production by supplementing an all alfalfa hay ration with 6 pounds of corn starch or with 6 pounds of corn sugar per day\**

Cow No.	Experi-mental period, days	In milk, days†	Body weight, lb.	Milk, lb.	Test, %	Fat, lb.	F.C.M., lb.	Alfalfa consumed, lb.	T.D.N.		Remarks
									Rec., lb.	Req., lb.	
Corn starch											
D14	15	145	1233	28.8	3.4	0.97	26.0	44.3	21.1	18.0	Alfalfa alone
	15	160	1212	28.0	3.1	0.88	24.4	42.1	25.3	17.3	“ plus starch
76	15	86	827	19.8	3.9	0.76	19.4	29.7	14.1	13.0	Alfalfa alone
	15	101	828	20.0	3.6	0.72	18.9	28.1	18.7	12.8	“ plus starch
237	12	165	1118	16.1	4.2	0.68	16.6	39.4	18.1	14.1	Alfalfa alone
	15	177	1136	19.2	3.8	0.73	18.7	36.7	22.0	14.9	“ plus starch
239	15	38	1145	27.0	3.6	0.95	25.1	36.1	15.6	17.0	Alfalfa alone
	21	53	1156	28.1	3.6	1.01	26.5	35.9	21.8	17.6	“ plus starch
Corn sugar											
266	15	185	1254	26.8	3.8	1.02	26.0	44.8	21.3	18.1	Alfalfa alone
	21	200	1277	25.8	3.9	0.99	25.2	42.7	25.8	18.0	“ plus sugar
A7	12	185	1202	19.9	3.1	0.62	17.2	40.0	18.5	14.9	Alfalfa alone
	12	197	1260	21.3	3.3	0.70	18.9	39.6	23.8	15.7	“ plus sugar
A22	15	88	1020	27.4	3.2	0.88	24.2	36.5	19.7	15.9	Alfalfa alone
	15	103	1015	27.1	3.3	0.90	24.5	28.2	20.0	15.9	“ plus sugar
D9	15	194	1188	16.0	3.8	0.60	15.4	36.8	17.8	14.2	Alfalfa alone
	15	209	1157	17.2	3.7	0.63	16.4	26.3	17.3	14.3	“ plus sugar
77	15	44	773	23.0	4.9	1.13	26.2	27.4	14.8	14.8	Alfalfa alone
	15	59	790	20.8	4.7	0.99	23.1	21.0	15.9	13.9	“ plus sugar
266	15	218	1222	21.1	3.6	0.77	19.9	39.7	20.4	15.9	Alfalfa alone
	15	233	1222	20.4	3.8	0.78	19.8	37.0	23.9	15.8	“ plus sugar

\* The original data were compiled by 3-day periods whereas the above values represent the mean values obtained for each experimental period.

† At beginning of experimental period.

TABLE 3

*Effects obtained in milk production by supplementing an all alfalfa hay ration with various amounts of corn starch or of corn sugar followed by various amounts of sugar or starch or both\**

Cow No.	Experimental period, days	In milk, days†	Body weight, lb.	Milk, lb.	Test, %	Fat, lb	F.C.M., lb.	Alfalfa consumed, lb.	T.D.N.		Remarks
									Rec., lb.	Req., lb.	
D14	30	7	1271	37.1	3.0	.....	31.4	34.4	17.2	19.9	Alfalfa alone
	30	37	1228	33.4	3.1	.....	29.1	35.0	22.5	19.3	" " plus 6 lb. starch
	15	67	1237	33.2	3.1	1.05	29.1	35.0	24.6	19.1	" " 9 lb. sugar
267	15	16	1029	27.3	3.4	0.93	24.8	36.5	17.5	16.2	Alfalfa alone
	9	31	977	24.8	3.3	0.83	22.3	35.7	20.1	15.0	" " plus 3.5 lb. starch
	6	40	962	23.1	2.7	0.63	18.7	37.5	23.9	13.7	" " 3.5 lb. starch
76	15	27	791	27.2	4.8	1.30	30.3	26.1	12.5	16.3	Alfalfa alone
	9	42	746	26.7	4.0	1.06	26.6	27.2	17.0	14.8	" " plus 4 lb. starch
	6	51	750	25.3	4.0	1.01	25.2	26.5	19.6	14.5	" " 4 lb. starch
77	15	35	725	22.9	5.0	1.14	26.2	25.0	12.0	14.5	Alfalfa alone
	9	50	680	20.4	4.9	1.00	23.2	27.1	17.5	13.2	" " plus 4.5 lb. starch
	6	59	691	20.0	4.4	0.88	21.1	26.5	20.2	12.6	" " 4.5 lb. sugar
77	12	121	774	18.0	5.1	0.93	21.1	26.3	12.5	13.2	Alfalfa alone
	12	133	768	17.7	4.9	0.87	20.2	26.4	18.1	12.9	" " plus 6 lb. sugar
	12	145	767	16.9	4.8	0.82	19.0	27.6	18.4	12.5	" " 6 lb. starch
A18	18	3	894	20.6	4.1	0.85	22.0	28.2	13.4	14.2	Alfalfa alone
	12	21	900	22.3	3.4	0.76	20.3	26.9	15.5	13.8	" " plus 3 lb. sugar
	12	33	889	20.9	3.0	0.62	17.7	26.9	20.6	12.8	" " 3 lb. sugar

\* The original data were compiled by 3-day periods whereas the above values represent the mean values obtained for each experimental period.

† At beginning of experimental period.

TABLE 4

*Effects obtained in milk production by supplementing an all alfalfa hay ration with 6 pounds of corn starch or with corn sugar followed with 6 pounds of corn or wheat per day\**

Cow No.	Experi-mental period, days	In milk, days†	Body weight, lb.	Milk, lb.	Test, %	Fat, lb.	F.C.M., lb.	Alfalfa consumed, lb.	T.D.N.		Remarks
									Rec., lb.	Req., lb.	
Corn starch											
A15	15	27	1133	31.3	3.0	0.93	27.3	34.7	17.9	17.5	Alfalfa alone
	15	42	1155	28.3	3.1	0.87	24.4	32.8	22.3	16.9	" plus starch
	18	57	1119	31.5	3.2	1.02	27.8	30.0	20.3	17.8	" " corn
A22	15	188	1032	18.4	3.6	0.66	17.2	40.0	20.2	13.7	Alfalfa alone
	15	203	1035	18.1	3.5	0.63	16.8	39.2	25.1	13.6	" plus starch
	18	218	1024	21.1	3.4	0.71	19.2	40.0	25.1	14.4	" " corn
A31	15	134	782	17.1	3.5	0.59	15.7	27.8	12.8	11.5	Alfalfa alone
	15	149	792	18.4	3.5	0.64	16.9	24.6	16.7	12.0	" plus starch
	27	164	811	22.3	3.5	0.79	20.7	24.7	16.2	13.3	" " corn
76	15	49	792	22.0	3.3	0.74	19.8	25.0	12.6	12.9	Alfalfa alone
	51	64	794	22.8	4.1	0.93	23.2	25.0	17.9	14.0	" plus starch
	18	115	841	22.1	4.5	1.00	23.8	25.0	17.5	14.5	" " wheat
237	15	118	1143	21.2	3.6	0.76	19.8	40.0	20.2	15.5	Alfalfa alone
	15	133	1147	19.9	3.4	0.68	18.1	39.0	25.1	14.9	" plus starch
	21	148	1139	20.7	3.8	0.78	20.0	35.0	22.5	15.4	" " wheat
Corn sugar											
A15	15	137	1034	24.6	3.6	0.87	23.0	44.8	20.7	15.6	Alfalfa alone
	15	152	1040	27.3	3.4	0.92	24.8	43.0	25.5	16.2	" plus sugar
	18	167	1060	32.7	3.3	1.06	29.3	44.1	25.2	17.7	" " corn
A18	15	134	1179	25.9	3.6	0.92	24.1	39.8	18.4	17.0	Alfalfa alone
	15	149	1175	24.8	3.7	0.92	23.7	33.9	20.5	16.8	" plus sugar
	15	164	1169	27.5	3.5	0.97	25.5	34.4	20.7	17.3	" " corn
266	15	44	1191	37.5	3.3	1.22	33.3	42.1	21.7	20.1	Alfalfa alone
	18	59	1210	41.4	3.2	1.33	36.5	38.0	24.4	21.2	" plus sugar
	15	77	1227	43.7	3.2	1.38	38.2	35.8	23.1	21.9	" " corn
266	15	131	1235	25.9	4.1	1.06	26.3	43.5	22.4	18.1	Alfalfa alone
	15	146	1215	25.8	3.9	1.02	25.6	36.0	23.4	17.6	" plus sugar
	15	161	1223	30.5	3.3	1.00	27.2	33.9	22.3	18.3	" " corn

\* The original data were compiled by 3-day periods whereas the above values represent the mean values obtained for each experimental period.

† At beginning of experimental period.

ward trend in milk production, fat and test after the alfalfa hay ration was supplemented with starch or glucose or a combination of both. Increasing the amounts of these supplements in the ration did not influence the natural decline in milk production with advancing lactation. Insofar as starch and glucose are concerned they were unable to change the course of lactation in these cows. Hay consumption was decreased slightly after the starch or glucose was added to the ration (average—0.3 pounds per day). When the amount of starch or glucose was increased or combined, there was a further reduction in hay consumption (average—0.3 pounds per day) over the preceding trial.

The results obtained from supplementing the alfalfa hay ration with 6 pounds of starch or glucose and then substituting 6 pounds of corn or wheat for the starch or glucose are presented in table 4. The addition of starch resulted in a decline in 4 per cent fat-corrected milk in 3 out of 5 trials. The fat production and percentage test followed the fat-corrected milk. When the starch was replaced with 6 pounds of corn or wheat, the fat-corrected milk increased against the tide of declining lactation in all trials. The average increase was 2.4 pounds per day over the starch feeding period and 2.3 pounds per day over the all alfalfa hay ration. The average of a 15-day period when either corn or wheat was fed does not do justice to the fat-corrected milk values reported in table 4 because about 6 days were usually required for the cows to reach the peak of production after the cereal grains were fed. The fat production and the test again followed the fat-corrected milk. The average hay consumption declined 1.4 pounds per day after the starch was added to the ration and decreased 1.2 pounds per day further after the corn or wheat replaced the starch supplement.

The addition of glucose to the alfalfa ration resulted in a decline in 4 per cent fat-corrected milk in 2 out of 4 trials. When glucose was replaced with 6 pounds of corn, milk production, fat and test increased in all trials. The average increase in fat-corrected milk was 2.4 pounds per day over the glucose feeding period and 3.4 pounds per day over the all alfalfa hay ration. The average daily hay consumption for this group of cows decreased 4.8 pounds after the glucose supplement was added to the ration and decreased 0.7 pounds per day further when corn replaced the glucose supplement.

The results obtained with Cow 266 (table 4) are of interest. When 6 pounds of glucose were added to the hay ration on the 56th day of lactation, the average daily increase in fat-corrected milk was 3.2 pounds for the 18-day period. Later in the same lactation period (149th day), the addition of 6 pounds of glucose resulted in a decrease in fat-corrected milk. In all probability the increased production of milk during the first trial was due to the carry-over effect from the dry period. In other words, the cow had not been depleted of the milk-stimulating factor or factors.

It is evident from all of these trials that supplements of starch and glu-



cose are ineffective in producing marked increases in milk production in cows on an all alfalfa hay ration. The replacement of these supplements with either corn or wheat, however, results in an appreciable increase in 4 per cent fat-corrected milk. The fact that both corn and wheat stimulated milk production against the tide of declining lactation suggests that these cereals furnish a factor or factors other than digestible calories. Milk production decreased in 9 trials and increased in 4 trials when starch supplemented the hay ration. The average decline in fat-corrected milk was 0.81 pounds per day. Milk production decreased in 7 trials and increased in 5 trials when glucose supplemented the hay ration. The average increase in fat-corrected milk was 0.02 pounds per day. The fat production and the test followed the fat-corrected milk very closely in nearly all cases. The average body weight changes varied less than  $\pm 1$  per cent for all of the trials. The alfalfa-starch ration caused a slight decrease in body weight, whereas, the alfalfa-glucose ration caused a slight increase. The changes in body weight, however, were insignificant. The addition of 3 to 9 pounds of starch or glucose to the alfalfa hay ration did not produce a significant change in alfalfa consumption although the dry matter intake was increased. Hay consumption decreased in 9 trials, increased in 3 trials and was unchanged in 1 trial. The average decrease in hay consumption on the alfalfa-starch ration was 0.8 pounds per day. Hay consumption decreased in 11 trials and increased in 1 trial on the alfalfa-glucose ration. The average decrease was 4.2 pounds per day. Dry matter intake increased when the starch and glucose supplements were fed to the cows but the starch-fed cows showed a greater increase than the glucose-fed cows.

#### DISCUSSION

It has been reported previously (17, 18) that the replacement of an equal amount of total digestible nutrients in alfalfa hay with corn in the ration of lactating cows, which had been depleted on alfalfa hay alone, resulted in a marked increase in milk production against the tide of decline in lactation. Supplementing the all alfalfa hay ration with cystine had little influence on lactation (18). This study shows the effects of supplementing the all alfalfa hay ration with calories fed in the form of corn starch or corn sugar. Kellner and Köhler's (24) early work indicated that the addition of starch to a maintenance ration of steers resulted in good utilization of starch for gain in fat. Our results show that starch and glucose failed to influence milk production significantly in cows that had been depleted of their milk-stimulating factors. When the cows had not been entirely depleted on alfalfa alone or when ketosis was involved, the feeding of starch and glucose stimulated milk production.

In table 4, cows A15, A22 and A31 produced 3.4, 2.4 and 3.8 pounds of 4 per cent fat-corrected milk per day more when 6 pounds of corn re-

placed 6 pounds of starch. Significant increases in milk production were obtained also when 6 pounds of wheat replaced the starch. The total digestible nutrients received during the corn- and wheat-feeding periods were the same for 3 cows but were slightly less for the other 2 cows. Cows A15, A18 and 266 produced 4.5, 1.8, 1.7 and 1.6 pounds of 4 per cent fat-corrected milk more per day when 6 pounds of corn replaced 6 pounds of glucose. The total digestible nutrients received during the corn-feeding periods were about the same as during the glucose-feeding periods. These data indicate that corn furnishes some factor or factors, other than calories, needed to balance an all alfalfa hay ration.

The productive energy value of feeding stuffs depends on the balance of the ration. Many authorities have used either the total digestible nutrients system or some method of calculating the so-called "productive energy," "net energy" or "starch value" of feeds. The starch value system suggested by Kellner (24) was based on his work with a respiration calorimeter. These values indicate that a pound of total digestible nutrients in concentrates was better utilized than an equal amount in roughage. Forbes *et al.* (9) observed that the metabolizable energy of a ration of alfalfa hay and corn was much better utilized than alfalfa hay alone. Fraps and Carlyle (10), using a corn and casein replacement method, concluded that the ability of the chicken to utilize the digested material is quite uniform. When the balance of the ration is taken into consideration there appeared to be little difference in the productive power of the digestible nutrients for fattening.

The data presented in this paper indicate that the reason for the low productive power of the all alfalfa hay ration was not due to a lack of digestible calories but that the inferior value of the total digestible nutrients in the alfalfa was due to a lack of the necessary dietary factors. Corn and wheat apparently furnish dietary factors other than calories which are not supplied in sufficient quantities in alfalfa hay for lactation. The data in the literature suggest that early cut hay is higher in the unknown factors supplied by grain than later cut hay.

The increase in milk production obtained by replacing starch or glucose with corn or wheat may be due to presence of a more favorable food for the microorganisms in the rumen. Mills and associates (28) found no evidence of increased microbiological activity in the rumen contents when starch was added to a ration of timothy hay. When urea and starch supplemented the hay, a suitable substrate was provided for the development of an active flora. There is a possibility that the flora and fauna in the rumen of the cows fed alfalfa alone were unable to synthesize the dietary factors needed for optimum milk production. The work of Hunt *et al.* (20) indicates that there was no riboflavin synthesized in the rumen of the steer on alfalfa hay alone but that synthesis increased as the amount of corn in the ration increased. Ground corn appeared to have a more favorable effect than whole corn. The amount of carbohydrate in the ration may stimulate riboflavin synthesis.

The feeding of starch as a supplement to an all alfalfa hay ration did not depress, the appetite for hay significantly under the conditions of this experiment. The average daily consumption of alfalfa alone was 33.8 pounds against 33.0 pounds when starch was added to the ration. The cows which received glucose consumed 33.3 pounds of alfalfa daily and 37.5 pounds on alfalfa alone. These data suggest that glucose tends to depress the appetite for hay to some extent but that starch has very little effect on hay consumption. The effect of glucose on appetite may be explained on the basis of rapid absorption from the rumen or, since this sugar is highly soluble, a large portion may have been washed rapidly through the true stomach and then absorbed from the small intestine. Phillipson and McAnally (32) demonstrated that the administration of glucose or cane sugar to a fistulated sheep caused an intense fermentation and a rapid fall in pH in the rumen during the first hour. No such reaction occurred after the administration of starch or cellulose.

#### SUMMARY

1. The effect of supplementing an all alfalfa hay ration with either corn starch or corn sugar (glucose) was studied on 25 feeding trials with 14 cows. Six pounds of starch or glucose were usually added per day.
2. The addition of starch or glucose to the alfalfa ration did not increase the production of fat-corrected milk although the total digestible nutrient intake was increased appreciably.
3. The replacement of 6 pounds of starch or glucose with 6 pounds of corn or wheat increased the production of fat-corrected milk markedly although the total digestible nutrient intake was not increased.
4. The addition of starch to the alfalfa ration did not reduce the appetite for hay but the addition of glucose tended to depress hay consumption.
5. The results of these experiments indicate that digestible calories are not the limiting factors in alfalfa hay for optimum milk production but that an unknown milk-stimulating factor or factors are supplied by corn or wheat when fed as a supplement to an all alfalfa hay ration.

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## A STANDARD FOR EVALUATION OF DAIRY SIRES PROVED IN DAIRY HERD IMPROVEMENT ASSOCIATIONS\*

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Dairy cattle are bred primarily for milk production, a character manifested in only the female sex. The males, however, carry the genetic factors determining ability to produce milk and the male parent exerts an influence essentially equal to that of the female upon the productive ability of the female offspring. Due to the relatively large number of offspring he leaves in the herd, the selection of the herd sire is a matter of great consequence. Since an outward expression of inherent productive ability is lacking in the bull, this must be evaluated largely upon the basis of the performance of related females. It is generally recognized that the future breeding worth of a dairy sire can best be evaluated on the basis of progeny which have reached producing age. There are many practical limitations to the general use of progeny-tested sires but in evaluating a pedigree some of the most valuable information is secured from the performance of progenies of the male ancestors. Consequently, proper evaluation of progeny tested bulls must be recognized as a matter of great importance in the breeding of dairy cattle.

Unfortunately, even though a bull may have daughters which have been tested for production, evaluation of his transmitting ability is not easy and predictions of his future breeding worth are likely to be of questionable reliability. Environmental conditions make tremendous differences in the degree to which a cow can express her inherited ability to produce. While reasonably satisfactory correction factors have been developed for some of these, there remain many non-genetic influences which cannot be equalized, and which introduce an element of uncertainty into any rating based upon production records. The principal problems of evaluating a bull on the basis of production of his progeny arise from the fact that he contributes only half of the gene complex of his offspring. Unless the contribution of the female parents can be correctly estimated, such evaluation on the sire has limited value. Many workers in the field of dairy cattle breeding have endeavored to arrive at a single index or a figure representing the potential productive

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ability of a progeny tested sire. Lush (2) has presented a very comprehensive discussion of the sire index question. The most commonly used and perhaps the oldest of these proposed indexes is based on the assumption that the offspring will tend to be intermediate in productive ability between that of the dam and the potential productive ability of the sire. Such a tendency is observed in the case of characters determined by multiple genetic factors and has been recognized for many years. Having a measure of any two of the animals of the parent-offspring trio, the productive ability of the third can be estimated. Ordinarily the male parent is the unknown of the equation, but once his capacity is established, his future daughters become the unknown in considering the use of the bull in a program of breeding. The most common form of this index, assuming exact intermediate inheritance, is known under various names such as equi-parent, equal parent, mid-parent, or intermediate index. A number of other indexes have been suggested, for the most part trying to make allowances for the unreliability of the production of the dams as a basis for estimating their contribution to their offspring and for the tendency for regression toward the breed average.

Theoretically, a satisfactory sire index should rate the bull the same, regardless of the productive level of the dams of his progeny, since the purpose of the index is to eliminate the influence of the dams and measure that of the sire alone. That the commonly used indexes fail in this respect has been concluded by a number of investigators from studies in which progenies of sires with large numbers of offspring were divided and the index was calculated separately on each portion of the progeny. It was found in each case that there was less variation between the simple averages of the progenies of the two groups than between the indexes calculated from comparisons with the dams. On this basis it has been suggested that the best measure of a bull's breeding worth is a simple average of the production records of his daughters, with due correction for non-genetic factors. This should not be interpreted, however, as meaning that the dams' records have no value for improving the accuracy of evaluation of breeding worth of progeny tested sires. It has repeatedly been shown that there is a definite correlation between records of daughters and of dams.

It is to be observed in those studies in which the dams were divided on the basis of productive levels there was a tendency for the average production of daughters from the higher producing dams to be above that of the daughters from the lower producing dams. This lends support to the belief that the records of the dams do have potential value in evaluating progeny tested sires, but that they may not be properly employed in the indexes studied, introducing greater errors than the error of disregarding entirely the information on the dams. In the studies cited, the method of dividing the progeny according to production of dams is subject to criticism, since the dams of the higher level will tend to include those favored by environment



and the errors of sampling as well as those genetically superior. Furthermore, it is highly probable that in a majority of the cases all or most of the daughters occurred in the same herd and that they and their dams were exposed to similar environmental conditions. Under such circumstances it is reasonable to expect that future daughters of the sire, from the same or closely related dams and exposed to very similar environment would resemble closely the previous daughters in productivity, and that any inherent errors of proposed indexes would reduce rather than improve the accuracy of a prediction of production of these future daughters. Certainly the simple average of the daughters' production would have very limited value in predicting production of future daughters under different environment. Nor would the equi-parent or similar indexes be expected to be much more satisfactory under such conditions.

The fact that the genetic make-up of the cows to which a bull is mated will influence the production of his daughters is axiomatic. Any index which takes no account of the contribution of the female parents is obviously omitting a matter of very serious consequence which, if it could be evaluated properly, would add to the accuracy of the final index. Unfortunately no entirely accurate measure of the contribution of the female parents is available, but this fact should not discourage the use of any measure which would materially increase the accuracy of the estimate.

It has long been recognized that the progeny of cows above the average productive ability of the breed tend to produce above the breed average but less than their dams, and that daughters of cows below the breed average tend to produce below the breed average but not as far below as their dams. Formulas for estimating expected performance of daughters on the basis of performance of dams have been worked out from various data and reported by different workers. This tendency of regression toward the breed average has been taken into account in the derivation of some of the formulas proposed for use in arriving at an index of breeding worth of proved dairy sires. While some of these appear to have definite merit, they have failed to meet with popular favor, possibly because they are not fully understood by those not mathematically inclined.

Norton (3) has suggested an index based upon this principle, but given in terms easily understood by the average breeder. He selected from the Holstein Advanced Registry, those cows whose dams also had advanced registry records. Their records for fat yield and those of the dams were corrected for age, number of milkings, and length of record. The daughters were then grouped according to the average butterfat production of the dams and an "expectancy table" was worked out showing the average production of daughters from dams of the various levels. This table is used as the basis for a proposed sire index. Instead of comparing the average production of the bull's progeny to that of their dams, Norton proposed

that it be compared to the expected production as indicated in his table of average experience or expectancy. He proposed that the difference between actual and expected production of the daughters be added to if greater, or, if lower subtracted from the average production of the daughters. This is the same as adding to the expected production, twice the deviation from expectancy, or an application of the equal parent principle with the expected production as a base.

The fundamental idea of the expectancy table seems very sound and practical, applying the principle of regression in a workable and easily understood manner, but the application suggested by Norton does not appear entirely logical. The production of the daughters, and the expected production, are influenced by the dam as well as the sire, and when the deviation from expected production is added to the production of the daughters, a rating is secured from which the influence of the dams has not been excluded, and which is not directly comparable to a rating secured on dams of a different average level of production.

Since a large number of sires is included, it is probable that the sires throughout the group will on the average be genetically approximately an average group of bulls for the breed. Moreover, it seems logical to assume that the average bull of a breed will have a genetic make-up of factors for productive ability essentially equivalent to that of the average cow. Therefore, it might be assumed that a bull whose daughters on the average reach only the level of the expectancy table is a bull with the average genetic make-up of his breed and that his potential productive ability is equivalent to the average production of females of the breed. The soundness of this assumption is borne out by the fact that the point at which the daughters average the same as the dams agrees closely with the average of the entire group. Below this level, the daughters tend to be superior to their dams, but above this level the daughters tend to be inferior, suggesting that the average effectiveness of the selection of bulls has not been very great.

It would, therefore, seem reasonable, if an index is desired expressing the potential productive ability of a sire under average environment based on the equal parent assumption, to double the deviation of his daughters from expectancy and add to the breed average for potential performance of sires, represented by the level at which daughters and dams are equal. This index is based on a definite environmental level, with the influence of the dams standardized, or merely Norton's index transposed to an average set of conditions. This treatment renders the index more suitable for comparing directly, ratings on different sires. Even if no attempt is made to arrive at a single expression of the sire's potential productive ability, the expectancy table should be exceedingly valuable as a practical standard of comparison or a "par" value by which to judge proved sires.

The publication of the results of the Nation-wide survey of dairy sires

proved in dairy herd improvement associations by the Bureau of Dairy Industry of the United States Department of Agriculture has provided a large volume of data from which expectancy tables may be derived for our five major dairy breeds under average Dairy Herd Improvement Association conditions. Being based upon progeny groups rather than individual daughter-dam pairs it should be even more useful as a standard for evaluating sire performance. It is felt that a table of average performance of this large group of sires, arranged by breeds, and according to average level of dams for milk yield, fat yield, and fat percentage should provide a very

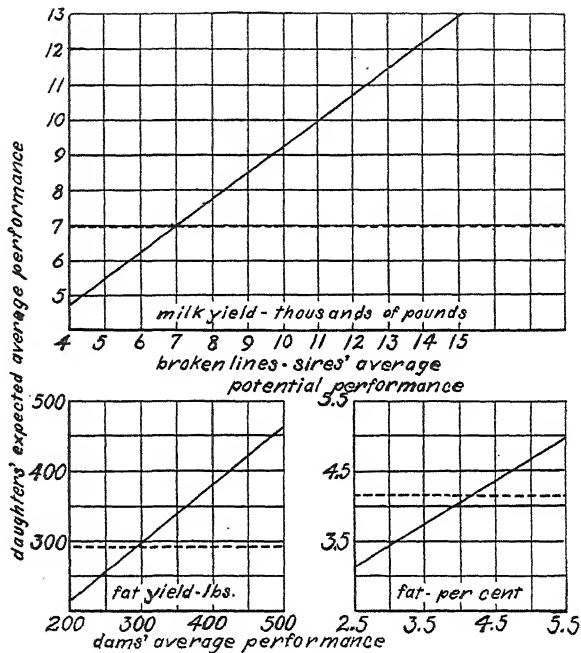


FIG. 1. Average performance of 214 Ayrshire sire progeny groups as related to level of performance of their dams.

valuable standard of comparison for evaluating proved sires. With this thought in mind, this study was undertaken.

The Division of Dairy Herd Improvement Investigations of the Bureau of Dairy Industry of the United States Department of Agriculture (1) has compiled the data gathered in the Nation-wide program of proving dairy sires which has been carried out in cooperation with the dairy extension service in the various states. This has made available daughter-dam comparisons for 5886 sires of the five major dairy breeds and provides a wealth of data for study of the performance of dairy sires under Dairy Herd Improvement Association conditions. The data for each breed have been con-

sidered separately. Duplication of sires has been avoided, using only the latest or most complete data for sires appearing in more than one of these published lists.

These data have been assembled according to the average performance of the dams to which the individual sires were mated. Milk production, fat production, and fat percentage have been considered separately. From these data for each breed and for each of the three performance factors the average level of performance of the sires' progenies has been estimated in relation to the average level of the dams to which they were mated. The

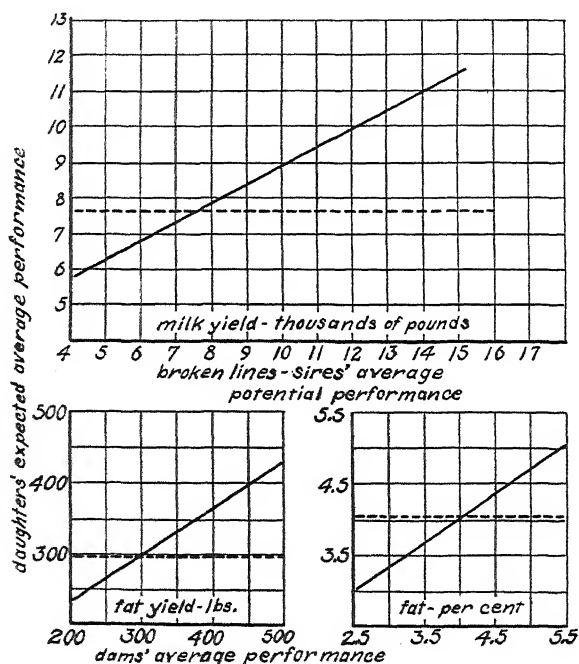


FIG. 2. Average performance of 124 Brown Swiss sire progeny groups as related to level of performance of their dams.

data have been fitted by the method of least squares and are presented graphically in figures 1 to 5 inclusive. In table 1 the comparative average performance of daughters and dams for the entire group is shown, together with the formula for estimating expected production of the progeny.

At the outset, it must be recognized that the progenies in the higher level of production will tend to have an average environment more favorable than those in the lower levels and the widest range of environment will fall in the median group. In all probability in the lowest levels are found the poorer animals genetically, and handled under the poorest conditions. In the highest levels will be found those with the highest genetic productivity

handled under the most favorable conditions. In the intermediate groups will be found animals of good heredity limited by poor environment and animals of relatively poor heredity but with favorable environment as well as animals of average heredity and average environment. It is also probable that more care has been exercised in selection of sires used on the dams of the higher levels of production and that there is some degree of hereditary superiority as compared to the sires used at the lower levels. The best prediction for a bull for continued use in the herd where he was proved should

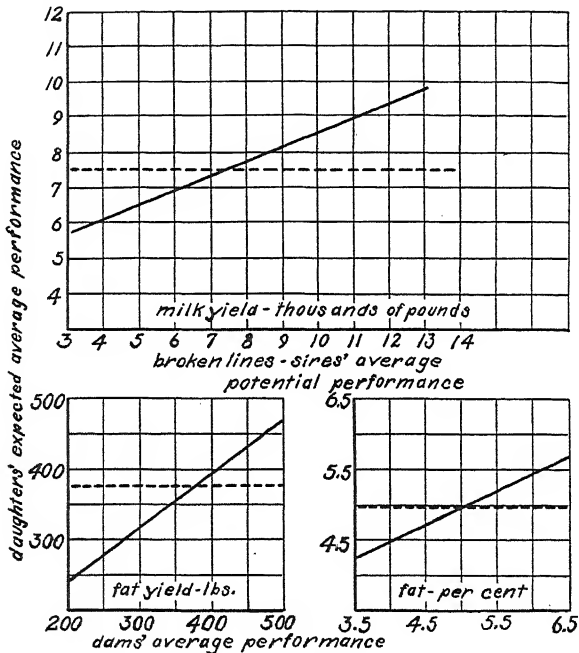


FIG. 3. Average performance of 1238 Guernsey sire progeny groups as related to level of performance of their dams.

be the simple average of his daughters, since his future daughters will be largely from the same group of dams and under very similar environment. Almost any index when used to forecast probable production of future daughters of the same dams would predict a production the same as for the previous daughters. For predicting his future daughters from a group of dams in another herd where both the hereditary contribution of the dams and the environment are different, a standard such as the expectancy formula given in this paper should have much greater value, provided the records are arrived at in the same manner as in those from which the standard is derived, *i.e.*, lifetime average, Dairy Herd Improvement Association, 305-day, mature, twice a day milking basis.

A study of the data in table 1 shows that except in the case of the Guernsey group, the level at which the daughters equal their dams in milk production is somewhat below the average for the breed. This suggests that the culling practiced in the cows is more effective than the selection of the sires, with either more effective selection of sires or less effective culling of cows, or both in case of the Guernsey group. Likewise in fat production the Holstein, Ayrshire, and Brown Swiss bulls failed to maintain production up to the breed average while the Guernsey and Jersey bulls maintained production slightly above the breed average. For butterfat percentage

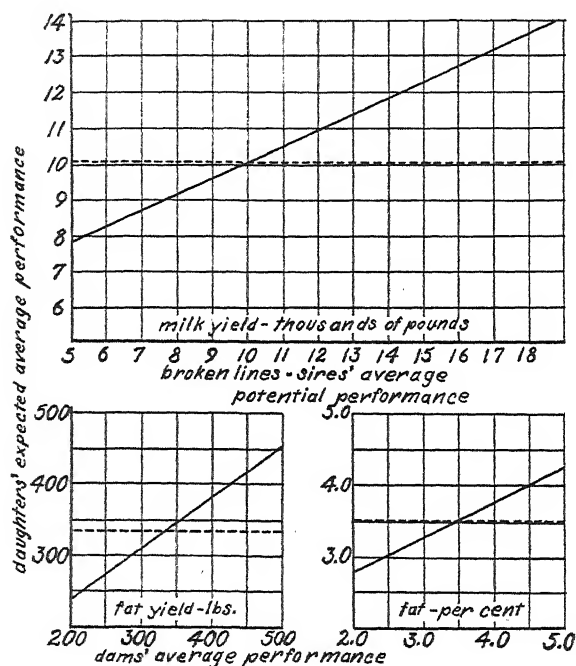


FIG. 4. Average performance of 3322 Holstein sire progeny groups as related to level of performance of their dams.

which is merely an expression of the relationship between milk yield and fat yield, in all breeds the sires were able to maintain a level higher than the average for the breed. This might be due to culling out the lowest milk producers among the cows. The poorest milk producers are likely to have a fairly high test due to an inverse relationship at lower levels of milk production.

The average milk production of the daughters is less than of the dams in all but the Guernsey breed, and average fat production is less in all except the Guernsey and Jersey breeds. The average production of the daughters will usually tend to be lower than of the dams in studies of large numbers

since the dams are influenced more by selection and culling. A cow that has a low yield for her first lactation is not likely to have a further opportunity to become a dam, since she will probably be culled from the herd. A situation in which the daughters equal the dams in average production over a period of years will result in a gradual increase in productivity for the population since such an increase will be necessary to mask the influence of differential selection and culling.

The correlation between daughters and dams is greater for fat yield than for either milk yield or fat percentage. This cannot be attributed entirely

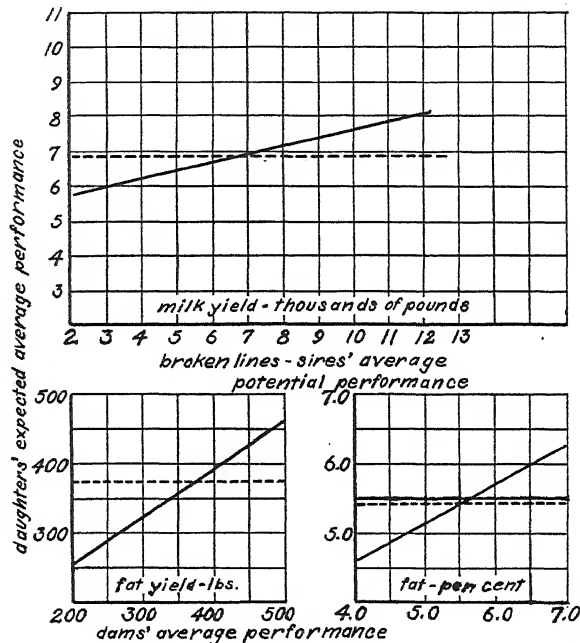


FIG. 5. Average performance of 988 Jersey sire progeny groups as related to level of performance of their dams.

to a greater degree of heredity as compared to fat percentage, however, since the daughters and dams tend to have similar environment and the fat percentage is influenced to a much lesser degree by environment. The Mount Hope index is applied to milk yield and to fat percentage. The equal parent index is in many cases likewise applied, the index for fat yield being the product of the indexes for milk yield and fat percentage. There are many reasons both genetic and physiological for believing that such indexes might more properly be applied to fat yield than to fat percentage.

Although the equal parent or any similar index will undoubtedly differentiate between extremes of potential productive ability, a relative rating

of two bulls based on the index alone with no knowledge of the figures from which the index was derived may be misleading.

While a modification of Norton's index has been suggested, based on a comparison of actual and expected production, it is not recommended that such an index be used in rating sires unless the data used in arriving at the index are included. A single figure such as this is apt to create an impression of greater accuracy than actually exists. It is felt that the greatest practical value of the expectancy tables lies in their use as a standard of

TABLE 1

*Relationship of production of daughters of sires proved in Dairy Herd Improvement Associations to production of their dams*

Breed	No. of sires	Average performance daughters	Average performance dams	Sires' average potential performance*	Formula for expected performance of daughters
Milk yield, pounds					
Ayrshire ...	214	7,821	8,103	6,980	1,752 + (.7490 × dams')
Br. Swiss ...	124	8,404	9,090	7,655	3,659 + (.5221 × dams')
Guernsey ...	1,238	7,530	7,462	7,578	4,427 + (.4158 × dams')
Holstein ...	3,322	10,414	10,769	10,135	5,672 + (.4403 × dams')
Jersey ...	988	6,935	7,160	6,865	5,226 + (.2387 × dams')
Fat percentage					
Ayrshire ...	214	4.035	3.964	4.144	1.658 + (.5998 × dams')
Br. Swiss ...	124	4.024	3.981	4.113	1.362 + (.6688 × dams')
Guernsey ...	1,238	4.872	4.787	4.951	2.563 + (.4825 × dams')
Holstein ...	3,322	3.473	3.436	3.510	1.775 + (.4945 × dams')
Jersey ...	988	5.294	5.195	5.415	2.435 + (.5503 × dams')
Fat yield, pounds					
Ayrshire ...	214	316.1	320.9	292.4	49.2 + (.8317 × dams')
Br. Swiss ...	124	338.1	361.4	296.7	106.6 + (.6407 × dams')
Guernsey ...	1,238	360.7	356.2	375.6	86.8 + (.7690 × dams')
Holstein ...	3,322	359.2	369.4	335.4	100.4 + (.7006 × dams')
Jersey ...	988	366.7	364.4	371.8	115.2 + (.6903 × dams')

\* The average potential performance of the sires is the level at which the average performance of the daughters equals that of their dams.

comparison by which to interpret the differences in production of progeny as compared to their dams. By the equal parent or similar indexes, when the bull is rated on the basis of extremely high producing dams, environment for both daughters and dams is almost certain to be very favorable. If this index is used to estimate the probable production of daughters in a herd of very low production where environment is apt to be very poor, the daughters will probably fall far short of the estimate. Likewise a bull used on dams of poor production, with daughters tested under the same environment, will probably be underrated for use in herds of high production. This



tendency might be offset to some degree by the greater assertion of the influence of dominant genes at lower levels of production. If a bull is rated on the basis of deviation of his daughters from expected production (using the expectancy tables for the breed as a standard) there may be a similar tendency but to a lesser degree since only the spread is affected. The base is a variable in which cognizance is taken of the difference in environment.

An index giving a specific rating on a bull is in itself of value only in making a choice between bulls. In the final analysis the factor of greatest importance is the accuracy with which production of future daughters may be predicted. This involves essentially a reversing of the process of calculating the bull's index. If this prediction is for future daughters in the same herd from the same dams, regardless of what index is used, the prediction will be the same as the average production of the daughters. For predicting production in a herd where environment and heredity of the dams are different, the index should make allowance for these differences. When used for predicting production of future daughters, the simple equal parent index does not make allowance for the differences in environment.

The suggested modification of Norton's index in which twice the deviation of daughters is added to the breed average for sires' potential performance should be more suitable for a comparative rating of sires, since environment is at least partially standardized and there is no regression at the level of production and environment for which the index is calculated. For calculating the probable production of future daughters, this index of the sire is not needed, only the deviation from expectancy being used. The average expected production may be estimated for the dams on which the bull is to be used. This gives a base in which differences both genetic and environmental are considered. To this base, the deviation of the bull's previous progeny from expectancy may be added to secure an estimate of probable production of future progeny. In doing this, a deviation below expectancy must, of course, be treated as a negative quantity. In the final analysis, this is nothing more than the equal parent index with compensation for differences in environment and for regression. By using the charts to determine expectancy, this method can be applied with no more calculation than a simple addition or subtraction.

Naturally, such a method will not fit all individual cases, but there is reason to believe that with no loss of simplicity, the average degree of accuracy should be greater than for the simple equal parent index. While no method gives a strictly accurate estimate, any method which increases the average degree of accuracy has practical value until a better is offered. These charts or formulas can be properly applied only to cases within the range of conditions of the population from which they were derived. For advanced registry conditions, a similar standard should be worked out from advanced registry data.

If accompanied by an adequate testing and record keeping program, artificial insemination associations should in a few years provide data ideally suited for a critical study of the comparative value of various bull indexes, with a possibility of arriving at a more accurate method of rating progeny tested sires than any yet suggested. While no method gives an entirely accurate estimate, any method which increases the average accuracy is desirable.

#### SUMMARY

1. From the lists of sires proved in dairy herd improvement associations, compiled by the Bureau of Dairy Industry, formulas have been derived for estimating the average expected production of daughters according to the productive level of the dams.

2. This average expected production of milk, of fat, and for fat percentage is presented in graphical form, to be used as a guide in evaluating progeny tested bulls and in predicting probable production of future daughters.

3. A modification of Norton's sire index is suggested for comparative rating of sires. Twice the deviation of the sire's daughters' production from the expected is added (negative deviations carrying a minus sign) to the breed average for potential performance of sire to give an estimate of the sire's hypothetical productive ability under average environment for the population.

4. To estimate probable production of future daughters, the deviation of his daughters' production from expected is added (deviations below expected being treated as negative values) to the expected production estimated for daughters of the cows to which he is to be mated.

5. This method applies the equal parent principle with a simple and workable allowance for regression and general differences of environment.

#### NOTE

Subsequent to the preparation of the manuscript for this paper, Rice (4, 5), has published a report of a study of the performance of Ayrshire sires whose provings were available in the records of the Ayrshire Breeders' Association. As a result of his investigation, he has suggested a method for evaluating progeny tested sires based on the same fundamental concepts as proposed in this paper.

Rice's proposed index differs in principle from that suggested in this paper in that he does not double the deviation from expected production before adding to the breed average. If the index is intended to represent the potential productive ability of the sire, the same principle of regression which is fundamental to the entire proposal suggests that this deviation should be doubled. In the simplified application based on fifty per cent

regression, this is identical to the ordinary equal parent index. As used by Rice, it differs by the amount of the deviation from expected production, being higher by this amount for bulls whose daughters fall below and lower for those whose daughters exceed expectancy. For predicting the performance of future daughters, which is the ultimate purpose of a sire index, the predicted production based on fifty per cent regression will be the same as if the simple equal parent method had been used, regardless of whether or not the deviation is doubled. This method of evaluating sires and predicting the performance of future daughters is an improvement over the ordinary equal parent procedure only to the extent that the regression varies from a value of fifty per cent, and any additional practical value which it may have is lost if the actual regression is rounded to fifty per cent.

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# THE RATIO OF ASCORBIC, NICOTINIC, AND PANTOTHENIC ACIDS, RIBOFLAVIN AND THIAMIN IN LATE SUMMER MILK\*

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Since milk serves as the sole nourishment of the young of the various species of animals that produce it, some have considered it a complete food, at least for young animals. Accordingly, many studies have been conducted to determine its nutritive value, and years ago data were accumulated concerning the minerals, protein, fat, and fatty acids which occur in cow's milk. During the discovery of vitamins or food accessory factors, as they were then designated, Hopkins (9), Osborne and Mendel (21), McCollum and Davis (15) and others found that cow's milk was a good source of the early recognized fat- and water-soluble vitamins. Langworthy and Holmes (13) found that milk fat was practically completely utilized by the human body. With the continuous accumulation of data concerning its nutritive value, the importance of milk as a human food has been constantly emphasized. However, recently additional water-soluble vitamins have been discovered and identified. Therefore additional data are required for a more complete evaluation of milk as a human food and particularly to enable one to judge the extent to which milk can be relied upon as a source of vitamins for bottle-fed infants and very small children whose diet consists very largely, and sometimes wholly, of milk. Accordingly, the present study was undertaken to accumulate data concerning the amount and ratio of ascorbic, nicotinic, and pantothenic acids, riboflavin and thiamin in cow's milk.

## EXPERIMENTAL

The milk used in this study was produced by the college herd. Since the herd consisted of seventy cows of five different breeds, Ayrshire, Guernsey, Holstein, Jersey, and Shorthorn, individual breed characteristics and other factors, such as age, stage of lactation and pregnancy, were largely, if not completely, eliminated. The season selected for conducting the study was late summer when the pasture grasses were fully mature, but before severe frosts had affected their quality. Inasmuch as there was an urgent demand for maximum milk production, the pasture ration was supplemented with ground grains (14% protein) at the rate of one pound for each six pounds of milk that the cows produced. The cows were all normal healthy animals, and were maintained under continuous veterinary supervision.

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The cows were milked by machine at 3:30 P.M. and 3:30 A.M. The afternoon milk was cooled at once to 40° F. and stored at that temperature until the morning milk was cooled and added to it. A sample of the combined afternoon and morning milk was taken at 8:00 A.M. when the average age of the milk was about ten hours. The assays were commenced at once upon the raw milk.

#### ASSAY PROCEDURES

The method described by Tripp, Satterfield and Holmes (26) and Holmes, Tripp, Woelffer, and Satterfield (8) was used to determine the ascorbic acid content of the milk. The nicotinic and pantothenic acids were assayed by Landy and Dicken (12) microbiological method using *Lactobacillus casei*. The milk samples were digested 24 hours at 45° C. with papain and clarase. The amounts of papain and of clarase used were 2 per cent of the weight of the sample. In computing the nicotinic and pantothenic acid content of the milk, allowance was made for the amounts of these vitamins in the enzymes. The riboflavin content of the milk was determined by the fluorescence method reported by Holmes, Jones, Wertz, and Kuzmeski (5). The thiochrome method of Hennessy and Cerecedo (3) was used in the assay of milk for its thiamin content.

#### RESULTS

##### *Ascorbic Acid*

The ascorbic acid in the fifteen samples of raw milk, table 1, varied from 17.1 to 20.3 mg. per liter and averaged  $18.4 \pm 0.69$  mg. This value is slightly less than 19.7 mg. per liter obtained by Holmes, Jones, Wertz, and Kuzmeski (5), for winter milk produced about eight months earlier by the same herd of cows, but it is higher than 17.3 mg. per liter reported by Holmes, Tripp, Woelffer, and Satterfield (8) for raw certified milk.

##### *Nicotinic Acid*

The nicotinic acid content of the milk varied from 0.96 mg. to 1.27 mg. per liter with an average of  $1.1 \pm 0.10$  mg. per liter. This value is higher than the 0.66 mg. value reported by Williams, Cheldelin, and Mitchell (30) and 0.6–0.9 mg. values found by Noll and Jensen (20) for skim milk. On the other hand it is lower than 1.46 mg. per liter reported by Bailey, Dann, and Satterfield (1) for Ayrshire milk produced in January, 3.0 mg. obtained by Kodicek (11) for fresh November and December milk, 1.2–4.4 mg. found by Melnick and Field (17), or 8.2 mg. per liter reported by Waisman and Elvehjem (27).

##### *Pantothenic Acid*

The amount of pantothenic acid in the milk ranged from 3.35 mg. to 4.10 mg. per liter with an average of  $3.66 \pm 0.31$  mg. per liter. The literature

consulted contained very little data concerning the pantothenic acid content of milk. Strong, Feeney, and Earle (25) found similar results, 4.0 mg. per liter, with those obtained in this study. Likewise, McElroy and Goss (16) obtained approximately the same value, *i.e.*, 4.5 mg. From their study, they concluded that pantothenic acid was synthesized by the microflora of the digestive tract for they limited the pantothenic acid intake of a cow to 17 mg. per day and obtained 41 mgs. daily in the milk that she produced. Jukes (10) reported that the whole milk he studied contained from 1.3 to 4.3 mg. with an average of 2.8 mg. of pantothenic acid per liter. Since milk forms the principal, if not the entire, diet of infants, it is interesting to compare these values for the pantothenic acid content of milk with those reported for Royal Jelly which is an important food for the very young of another species. Pearson and Burgin (22) report that Royal Jelly contains 179 mg. of pantothenic acid per 1000 gm., which is about forty-five times as much as was found in the milk used in this study.

TABLE 1  
*Vitamin content of late summer raw milk:*  
(Milligrams per liter)

Sample No.	Ascorbic acid	Nicotinic acid	Pantothenic acid	Riboflavin	Thiamin
1	18.5	.....	3.45	1.48	0.53
2	17.3	.....	3.95	1.29	0.45
3	18.3	.....	3.88	1.39	0.53
4	18.0	.....	3.55	1.31	0.43
5	17.5	.....	4.10	1.44	0.41
6	17.1	.....	4.05	1.43	0.44
7	17.9	1.27	3.35	1.40	0.43
8	17.8	1.00	3.37	1.32	0.41
9	18.8	1.01	3.85	1.34	0.42
10	18.8	1.05	3.47	1.31	0.45
11	18.3	0.96	3.27	1.32	0.41
12	20.3	1.14	.....	1.38	0.43
13	18.8	1.13	.....	1.31	0.44
14	18.8	1.14	.....	1.42	0.45
15	19.3	1.20	.....	1.42	0.44
Average	18.4 ± 0.69	1.1 ± 0.10	3.66 ± 0.31	1.37 ± 0.08	0.44 ± 0.04

### *Riboflavin*

The riboflavin content of the milk produced during the experiment varied from 1.29 mg. to 1.48 mg. per liter, and averaged  $1.37 \pm 0.08$  mg. Except for these extreme values the variation was only 0.14 mg. which indicates the milk was of quite uniform riboflavin content. The average value is somewhat lower than 1.51 mg. reported by Holmes, Jones, Wertz, and Kuzmeski (5) and 1.45 mg. to 1.50 mg. reported by Holmes and Holmes (4) for raw winter milk produced by the same herd. It is also lower than the values, 1.76 to 2.55 mg. of riboflavin per liter, reported by Whitnah, Kunerth, and Kramer (28), 7.8 mg. reported by Lindholm (14), and 1.5 to 2.5 mg. reported

by Snell and Strong (24), but the riboflavin content of milk obtained in this study is higher than the values of 1.0 to 1.2 mg. per liter reported by Randoin and Raffy (23), and of 0.95 mg., reported by Williams, Cheldelin, and Mitchell (30).

#### *Thiamin*

The thiamin content of the samples of milk studied varied from a minimum of 0.41 mg. per liter to a maximum of 0.53 mg. per liter. The average value obtained for the 15 samples was  $0.44 \pm 0.04$  per liter. This is identical with the value reported by Williams, Cheldelin, and Mitchell (30) for Jersey and Guernsey milk. A number of other investigators report somewhat lower results for the thiamin content of milk. Halliday and Deuel (2) found the average thiamin content of certified Holstein milk was 0.41 mg. per liter. Widenbauer and Heckler (29) obtained values ranging from 0.13 to 0.57 mg., and averaging 0.32 mg. per liter and Morgan and Haynes (18), found three samples of market milk contained 0.27, 0.30, and 0.32 mg. of thiamin per liter.

#### *Ratio of Vitamins in Milk*

Since cow's milk is a natural product that is extensively recommended and very generally used for human food, it is of interest to consider the ratio of vitamins in it. This is particularly the case for milk which is destined to be the principal and sometimes the whole source of nourishment for infants. The ratio of the five vitamins under consideration was computed for each of the fifteen samples of milk assayed, but since these data are rather voluminous, only the average values will be reported here. When the thiamin content of the milk was used as a basis of computation it was found that the ascorbic acid was 41.6, the pantothenic acid 8.2, the riboflavin 3.3, and the nicotinic acid 2.5 times larger. Using nicotinic acid as the basis of comparison, the ascorbic acid was 17.2, the pantothenic acid 3.3, the riboflavin 1.2, and the thiamin 0.39 times larger. Considering the riboflavin content of the milk as a basis, the ascorbic acid was 13.5, the pantothenic acid 2.7, the nicotinic acid 0.81, and the thiamin was 0.32 times as much. If pantothenic acid is used for comparison, the ascorbic acid was 4.2 times, riboflavin 0.28, nicotinic acid 0.26, and thiamin was 0.10 times the pantothenic acid content of the milk. Computed on the basis of the ascorbic acid in the milk, the amount of pantothenic acid was 0.21 times, the riboflavin 0.08, the nicotinic acid 0.06, and the thiamin was 0.02 times the amount of ascorbic acid found in the milk.

It is of interest to compare the ratio of the five vitamins in the milk under consideration, with the ratio of these vitamins recommended for infants. After extensive study by experts in the field of human nutrition, the Food and Nutrition Board of the National Research Council (19) adopted and recommended daily allowances of the various dietary essentials for



people of different ages. The recommended allowances for children under one year old are: ascorbic acid 30 mg., nicotinic acid 4 mg., riboflavin 0.6 mg., and thiamin 0.4 mg. daily, the ratio of the vitamins being ascorbic acid 75 times, nicotinic acid 10 times, and riboflavin 1.5 times that of thiamin. As stated above, the ascorbic acid content of the milk under investigation was 41.6 times, nicotinic acid was 2.5 times, and riboflavin was 3.3 times the thiamin content of the milk. Consequently, in order to modify this milk to meet the National Research Council's recommended dietary allowances for infant feeding, it is necessary to fortify it with natural or synthetic vitamins. The recommended allowance for ascorbic acid is 30 mg. daily, but the milk under investigation contained only 18.4 mg. per liter. Thus to obtain 30 mg. daily from the milk, an infant would have had to consume about one and two-thirds liters daily, a physical impossibility. Accordingly, it was a routine practice in infant feeding to supplement a modified cow's milk formula with orange, tomato, or other juices. Recently, however, because of the uncertain ascorbic acid content of oranges, as shown by Holmes, Pigott, and Tripp (7), Holmes, Patch, and Tripp (6), and others, hospitals and pediatricians have tended to supplement milk formulas with synthetic ascorbic acid since thereby a definite amount may be fed daily. One liter of the milk under discussion would supply exactly the amount of thiamin, twice as much riboflavin, but only one-fourth of the nicotinic acid recommended by the National Research Council for infants. Unfortunately, there are few natural foods suitable for infant feeding, which can be fed in sufficient amounts to provide the quantity of nicotinic acid required to fortify the milk under consideration to meet the vitamin allowances recommended by the National Research Council. Thus it appears that in order to modify the milk used in this study, which is doubtless typical of large volumes of commercial milk, so that it would meet the vitamin allowances recommended by the National Research Council for infant feeding, one must use foods exceedingly rich in nicotinic acid or a synthetic preparation of it.

#### SUMMARY

Fifteen samples of late summer milk produced under typical modern dairy farm conditions, were assayed for ascorbic, nicotinic, and pantothenic acids, riboflavin, and thiamin. The average vitamin content of the milk was ascorbic acid  $18.4 \pm 0.69$  mg., nicotinic acid  $1.1 \pm 0.10$  mg., pantothenic acid  $3.66 \pm 0.31$  mg., riboflavin  $1.37 \pm 0.08$  mg., and thiamin  $0.44 \pm 0.04$  mg. per liter. The ratio has been computed for each vitamin to the other four vitamins of the group. For instance, considered on the basis of the thiamin content of the milk, the ascorbic acid is 41.6 times, nicotinic 2.5, and riboflavin 3.3 times its thiamin content, whereas the recommended vitamin allowances for infants are in the ratio of ascorbic acid 75 times, nicotinic acid 10 times, and riboflavin 1.5 times that of thiamin. Hence the milk

under consideration would have to be fortified with ascorbic and nicotinic acids to meet the recommended allowances for infant feeding.

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## A NOTE REGARDING THE SPEEDS OF BABCOCK CENTRIFUGES

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When Stephen M. Babcock first described his test for fat in milk (1), he did not specify the exact speed at which bottles were to be centrifuged. In a later report (2), he said that the speed should vary between 700 and 1200 r.p.m. depending upon the diameter of the wheel, but he did not go into details.

Several years later, a table was published by others (3) which was supposed to show the speeds at which machines of various sizes should be operated to secure equal centrifugal forces. During the past forty-seven years, this table has been incorporated into most textbooks dealing with the subject, and into the Babcock regulations of many of the states, in spite of the fact that it is based upon a faulty assumption.

The faults of the present table become obvious when one considers the statement that a machine 10 inches in diameter (5-inch radius) is to be operated at a speed of 1074 r.p.m. As a standard test bottle is 6.5 inches high, the neck of the bottle would project 1.5 inches past the center of the machine, and any fat in this portion of the neck would actually be thrown out of the bottle by centrifugal force. Nevertheless, the regulations of at least seven states apparently authorize this procedure.

It should be stated that the authors of this table were undoubtedly aware of this situation. Their values for centrifuges of small radius are appropriate if very short bottles are centrifuged, such as those less than five inches high which are sometimes used for testing human milk. These criticisms are directed, not toward the table itself, but toward its misuse by the unwary who may not realize that the proper radius of the machine, and its proper speed, are dependent upon the height of the bottle which is to be centrifuged.

The table of speeds in common use was calculated to yield equivalent forces upon particles in the bottoms of the cups. The forces acting upon the material inside the bottle, or in the neck, are substantially less because they are nearer the center of rotation. Table 1 shows the relative forces acting at the bottom of the cup, at the fat-acid interface during the first (and principal) centrifuge period and at a point half way up the neck of the bottle, under the conditions which are commonly specified.

Obviously this table gives equal forces at the wrong place in the test bottle. However, it is not obvious which point in the test bottle should be used for these calculations.

It seems probable that the first centrifuging is the most critical of the three centrifuge periods. During the first period, the centrifugal force must

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TABLE 1

*Relative forces at different positions in the bottle when centrifuged at the speeds in common use*

Diameter	Speed	Relative forces exerted			
		At the bottom	1.5 inches above the bottom	At the middle of the neck	
				6-inch bottle	9-inch bottle
	<i>r.p.m.</i>				
10	1074	1	0.7	0.050	-0.200
12	980	1	0.75	0.208	0.000
14	909	1	0.786	0.321	0.143
16	848	1	0.813	0.406	0.250
18	800	1	0.833	0.472	0.333
20	759	1	0.850	0.525	0.400
22	724	1	0.866	0.568	0.455
24	693	1	0.875	0.604	0.500

act upon fat in the form of minute droplets and collect it into an oily layer. During the later periods, this oil layer is brought into the neck of the bottle, and much smaller forces are needed because of the larger sizes of the oil droplets. If this first centrifuging is the most important one, it would seem advisable to standardize the force acting at the top of the acid layer at that time. The forces acting below this surface would always be greater than the standard force, never smaller.

For this reason, it is suggested that speeds should be used which yield equal forces at a point 1.5 inches above the bottom of the centrifuge cups. These speeds are given in table 2. This table also shows the relative forces acting in each case at the bottom of the cup, and at a point half way up the neck.

TABLE 2

*Relative forces at different points in the test bottle when centrifuged at the proposed new speeds*

Diameter	Speed	Relative forces exerted			
		At the bottom	1.5 inches above the bottom	At the middle of the neck	
				6-inch bottle	9-inch bottle
	<i>r.p.m.</i>				
10	1171	1.428	1	0.071	-0.286
12	1033	1.333	1	0.278	0.000
14	934	1.272	1	0.409	0.182
16	859	1.230	1	0.500	0.308
18	800*	1.200	1	0.567	0.400
20	751	1.176	1	0.617	0.471
22	711	1.157	1	0.657	0.526
24	677	1.142	1	0.690	0.576

\* According to Farrington and Woll (3), Babcock's machine was 18 inches in diameter. Their table of speeds was calculated with the assumption that such a machine should operate at 800 r.p.m. This assumption was retained when calculating the speeds in this table.

It is apparent that the force in the neck of the bottle may be relatively low, especially in the machines of smaller diameter. It would seem undesirable to have this force become too small because of the slow separation of water emulsified in the fat column. There are no data available to indicate the minimum permissible force in the neck of the test bottle. However, the use of ten-inch and twelve-inch machines should certainly be discouraged; in fact, they have already been dropped in some states, and it might be advisable to discontinue the use of fourteen-inch machines, at least in the case of commercial and official testing. When the long-neck cream bottles are used, the use of even an eighteen-inch tester would appear questionable.

#### SUMMARY

The table of speeds of Babcock centrifuges in common use gives equal forces at the bottom of the cup, not in the bottle where the sample is placed. A new table has been calculated to produce equal forces at the fat-acid interface during the first centrifuging period. It is recommended that the use of small diameter centrifuges be discontinued because the force acting to remove water emulsified in the fat column is very small in the small machines.

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# THE VALUE OF KELP MEAL IN RATIONS FOR DAIRY CATTLE<sup>1</sup>

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Dried kelp has found considerable use as a source of minerals in livestock feeding. According to the producers of kelp meal, it contains a remarkably high content of some 30 odd mineral salts that are beneficial to the health of livestock, especially with respect to their reproductive functions, growth, and increased milk production. On the other hand, a limited number of available reports on the use of kelp meal, or kelp in combination with other products in proprietary mixtures, have shown no appreciable benefits from adding them to well balanced rations for dairy heifers and dairy cows (1, 2, 3, 4, 6).

Because of the claims made and the fact that many dairymen have purchased kelp meal, or mixtures containing kelp, it seemed practicable to conduct an investigation to obtain more information on the value of adding kelp meal to the rations of dairy cattle, and the results are herein reported. The kelp meal used was produced from the giant kelp (*Marcocystis pyrifera*) that grows in the Pacific Ocean off the coast of Southern California.

An average analysis of the meal made from the dried ground plant is as follows:

Ash (natural plant minerals) .....	35.2%
Carbohydrates (nitrogen-free extract) .....	42.9
Protein .....	5.6
Fat (ether-soluble) .....	0.5
Fiber .....	6.5
Moisture .....	9.3
	<hr/>
	100.0%

## EXPERIMENTAL

The original plan was to feed a group of heifers from an average age of 12 months to first calving on the usual roughages and a grain mixture containing 4 per cent kelp meal and to compare the results obtained with another group fed the same grain mixture without the kelp. During the progress of the investigation, however, the plan was modified to continue the animals through their second gestation period to obtain data for a second parturition. Also, this provided an opportunity to obtain lactation data.

Seventy-eight heifers (24 Holsteins, 24 Ayrshires, 18 Guernseys, and 12 Jerseys) were started on the experiment and 50 of these animals (18

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Holsteins, 18 Ayrshires, 8 Guernseys and 6 Jerseys) were continued through the second gestation period. When placed on the experiment, the heifers were paired according to breed, age, size and previous growth data. For the most part, the heifers of each pair were paternal half-sisters. They were free of known diseases and were examined for any genital abnormalities before breeding. All heifers were bred to freshen at 27 to 30 months of age.

The heifers received U. S. No. 2 clover hay and corn silage in amounts that would be readily consumed each day. They received no pasture. In addition, four pounds of a concentrate mixture containing approximately 14 per cent total protein were fed to each heifer daily. The grain mixture was as follows:

600 lbs. yellow corn meal  
600 lbs. ground oats  
500 lbs. wheat bran  
250 lbs. linseed meal  
30 lbs. steamed bonemeal  
20 lbs. salt

Heifers on the kelp ration received the same mixture to which was added four per cent kelp meal. The amount of concentrates fed was increased before calving, depending upon the physical condition of the individual heifer. About two weeks before calving a mixture of ground oats and wheat bran was substituted for the regular concentrate mixture. Four per cent of kelp meal was added to the oats and bran for the animals in that group.

During lactation, a grain mixture containing 16 to 18 per cent total protein was fed. This mixture contained one per cent each of bonemeal and salt. Four per cent of kelp meal was added to this mixture for the kelp-fed cows. The amount of grain fed to each cow was adjusted weekly on the basis of production during the preceding week. Grain was fed at the rate of 1 pound to each  $3\frac{1}{2}$  pounds of milk produced for Ayrshires and Holsteins and 1 pound to each 3 pounds of milk produced for Guernseys and Jerseys. Roughages consisted of U. S. No. 2 leafy alfalfa hay and corn silage. The cows were fed all they would consume readily of these roughages when fed twice daily. No pasture was available. All feeds were weighed and refused feed was accounted for in order to determine whether kelp meal had any effect on feed consumption.

## RESULTS

*Reproduction.* Growth and reproductive data are presented in table 1 for all heifers through the first gestation period.

As noted in table 1, gains in liveweight for both groups of heifers were practically equal, showing no advantage to either ration in this respect. Likewise, no favorable effect of kelp meal is shown in breeding efficiency. In the control group, 1.46 services were required for each conception, while

1.86 services per conception were required by the heifers receiving kelp. Sixty-five per cent of the control heifers conceived on the first service as compared with 54 per cent for the kelp animals.

There were two abortions in each group, five cases of retained placenta in the control group and two in the kelp group, and two heifers from each group were discarded as non-breeders. All of these non-breeders showed genital abnormalities which could not be attributed to the rations being fed. If an animal failed to carry her calf 260 days it was considered as an abortion.

Eliminating the animals which aborted, there were no differences in length of gestation between the two groups. No significant differences were

TABLE 1  
*Growth and breeding data on heifers through first gestation*

	Control ration	Kelp ration
Number of animals .....	39	39
Average daily gain (lbs.) .....	1.05*	1.03*
Services per conception .....	1.46*	1.86*
Service at which conception occurred:		
First (%) .....	65	54
Second (%) .....	30	22
Third or more (%) .....	5	24
Length of gestation (days) .....	276.5†	275.9†
Abortions .....	2	2
Retained placentas .....	5	2
Non-breeders .....	2	2
Av. deviation in wt. of calves at birth from normal (lbs.)	-3.3±	-1.09
Physical condition of calves at birth .....	29 Good	20 Good
	4 Fair	5 Fair
	0 Thin	0 Thin
	2 Poor	0 Poor

\* Does not include 2 animals from each group discarded as non-breeders.

† Does not include 2 animals from each group that aborted and 1 animal from control group for which date of service was not recorded.

noted in the condition of calves at birth, except that two small, weak calves were born in the control group. The average birth weights of the calves slightly favored the kelp-fed group. The normal birth weights used for comparison were those of Morrison (5). Weights below normal were to be expected since they were all from first calf heifers.

While the data on services required for conception tend to favor the control group, the number of abortions was the same in both groups and the physical condition of the calves at birth gives a small advantage to the kelp-fed group. Such differences are not great enough to be significant.

After part of the heifers had calved and were taken off the experiment, it was decided to continue the remaining animals on through their second gestation. This allowed the feeding of kelp through two consecutive gestation periods and makes it possible to study the effect of continuous feeding

of kelp meal. Such data are available on 25 pairs of animals and a comparison of the results for the first and second calvings is given in table 2.

When the results of the second gestation and calving period are compared with those of the first, no improvement is observed in breeding efficiency of the kelp-fed animals. Services per conception remained about equal for the second gestation as compared with the first and the physical condition of the calves at birth was essentially the same. The size of calves at birth was slightly lower on the kelp ration than on the control ration at second calving. At first calving, the reverse was true.

TABLE 2  
*Comparison of breeding records between first and second gestations*

	Control ration		Kelp ration	
	First gestation	Second gestation	First gestation	Second gestation
Number of animals .....	25	25	25	25
Services per conception .....	1.56	2.20	1.92	1.96
Service at which conception occurred:				
First (%) .....	60	52	56	44
Second (%) .....	32	8	20	28
Third or more (%) .....	8	40	24	28
Length of gestation (days) .....	276.9	278.8*	275.3	277.7*
Abortions .....	0	4	0	3
Retained placentas .....	1	2	1	4
Non-breeders .....	0	0	0	0
Av. deviation in wt. of calves at birth from normal (lbs.) .....	-1.08	+5.68†	0	+4.86
Physical condition of calves at birth .....				
	24 Good	21 Good	21 Good	20 Good
	0 Fair	0 Fair	4 Fair	2 Fair
	0 Thin	0 Thin	0 Thin	0 Thin
	1 Poor	0 Poor	0 Poor	0 Poor

\* Does not include 4 animals in control group and 3 animals in kelp group that aborted.

† Includes a pair of twins in control group.

The number of cases of retained placenta increased for the second calving as compared with the first calving for the kelp-fed animals. Also, there was a higher number of abortions in both groups. It is highly questionable if these abortions should be attributed to the experimental rations. The University herd has been free from Bang's disease, trichomoniasis and other genital infections, but an unusually large number of abortions occurred in the entire herd during the progress of this investigation. Apparently these abortions have been due to some other cause as yet undiscovered. It is worthy of note, however, that whatever the cause may be it was not improved by the addition of kelp meal to the ration.

*Milk production.* Lactation and feed records were kept for the first 305 days, except in a limited number of cases where the animals went dry before

milking that long. After the cows were dried off they were changed to the concentrate mixtures fed the heifers.

Feed consumption and lactation data are presented in table 3.

These records show no consistent differences which can be attributed to the rations fed. While a slightly higher production was obtained from the cows on the control ration, when the variability is considered, this difference is not significant and probably can be accounted for on the basis of individuality of the animals.

The addition of kelp meal had no stimulating effect upon appetite since feed consumption was essentially the same on both rations. The feed re-

TABLE 3  
*Average milk production and feed consumption during lactation*

	Control ration	Kelp ration
Number of animals .....	25	25
Av. age at freshening (yrs.) .....	2.47	2.51
Changes in liveweight (lbs.) .....	+ 44.9	+ 78.4
Length of lactation (days) .....	303.5	303.4
Milk production (lbs.) .....	8076.2	7537.6
Fat percentage .....	4.01	4.14
Fat (lbs.) .....	323.8	312.2
4% fat-corrected milk (lbs.) .....	8086.9	7698.2
Grain consumption (lbs.) .....	2487.6	2338.4
Silage consumption (lbs.) .....	9116.3	9170.3
Hay consumption (lbs.) .....	2887.2	2785.4
Grain per cwt. 4% F. C. M. (lbs.) .....	30.8	30.4
Silage per cwt. 4% F. C. M. (lbs.) .....	112.7	119.1
Hay per cwt. 4% F. C. M. (lbs.) .....	35.7	36.2

quired to produce 100 pounds of four per cent milk was almost identical for the two rations.

Growth during lactation, as measured by changes in liveweight, slightly favors the kelp ration, but the difference is not significant.

At no time during the progress of this investigation was it possible to detect any consistent differences in physical appearance of the animals on the two rations. Both groups maintained a good, thrifty condition.

#### DISCUSSION

These results do not show any special health or growth benefits from the addition of four per cent kelp meal to the grain mixtures of dairy heifers and cows. The normal ration contained steamed bonemeal as a source of calcium and phosphorus. It is possible, of course, that if the control ration had been very low in mineral content, *i.e.*, deficient in phosphorus, calcium or iodine, the results might have been more favorable to kelp meal. The primary objective, however, was to determine if the trace or rare minerals con-

tained in kelp would show any benefits after feeding for an extended period of time over those obtained from feeding rations generally considered satisfactory and containing the simple supplements, salt and bonemeal.

In some cases, claims have been made that beneficial results from kelp were not obtained because it was fed for an insufficient length of time. Because of this the experiment was continued through the second gestation and it would seem that 30 months of continuous feeding of kelp meal at the rate of four per cent in the grain mixture is sufficient for any effects to show up. It is appreciated that kelp and other marine products furnish iodine, and in sections where a deficiency of iodine manifests itself, the results might have been slightly different. Also, it is known that the iodine content of milk may be increased by feeding products rich in the mineral element. However, the results of this investigation confirm those previously reported in showing no benefits from adding kelp to well balanced rations for dairy heifers with respect to growth, breeding efficiency, and milk production.

#### SUMMARY

Feeding experiments have been completed with 39 pairs of heifers to determine the value of adding kelp meal to dairy rations. Kelp meal was fed at the rate of four per cent of the concentrate mixture to one animal of each pair.

Under the conditions of this investigation, the addition of kelp meal to normal rations for dairy heifers showed no particular benefits for growth, breeding efficiency, general health, physical condition, appetite, and size and condition of calves at birth.

Continued feeding of kelp meal through a second gestation showed no favorable effects on breeding efficiency, feed consumption, and milk production.

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# EFFECT OF COLOSTRUM ON THE VITAMIN A AND CAROTENE CONTENT OF BLOOD PLASMA OF NEW-BORN CALVES\*

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The literature on colostrum from the standpoint of the protection it affords the young by immunization against certain types of infections has been amply reviewed by Rogers and associates (8). Colostrum, however, contains vitamins and possibly many other substances which are necessary for the proper health of the new-born. Dann (1) found the vitamin A concentration in colostrum may be 10 to 100 times greater than in later milk from the same cow. He states "on the first day of life a calf receives supplies of vitamin A greater than the later milk could give in 20 to 50 days." Stewart and McCallum (12) found the vitamin A content of colostrum varied from 35 to 1181 I.U. per 100 ml. in samples collected from 100 cows. By the third or fourth day the amount of vitamin A is 1/10 to 1/20 that taken immediately after parturition. Semb and associates (9) found the carotene and vitamin A content of butterfat prepared from colostrum was from 5 to 15 times that of fat from ordinary milk. They emphasized the importance of colostrum to build up the vitamin A stores in new-born calves. Kramer and co-workers (3) reported values of 25 and 28 International Units of vitamin A activity per gram of colostrum for two cows receiving rye pasture and values of 16 and 20 International Units for two cows on winter rations.

Stewart and McCallum (14) were unable to increase the vitamin A content of colostrum by feeding 3 lbs. of carrots or 1/7 pint of codliver oil per day to cows on winter feed. These same authors (13) also made an extensive study of the correlation between the incidence of white scours in calves and the vitamin A content of the colostrum. In 83 calves which received colostrum containing more than 250 blue units of vitamin A, 10.8 per cent developed white scours or allied infections; whereas, in 28 calves which received colostrum containing less than 250 blue units of vitamin A, 25 per cent developed white scours or allied infections.

The effect of the intake of colostrum on the vitamin A and carotene content of the blood plasma of calves has not been widely studied. Phillips, Lundquist, and Boyer (7) have reported data on a few calves. Lundquist and Phillips (4) reported data on 45 calves on blood samples drawn at birth, the first, second and seventh days.

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Because of the practical importance of colostrum to the new-born and because all the factors concerned have not been evaluated, it was decided to study the effect of colostrum intake on the vitamin A and carotene content of blood plasma of new-born calves. The results of the study are reported herein.

#### EXPERIMENTAL

Blood samples were drawn from calves dropped in the University of Maryland herd of the Holstein, Ayrshire, Jersey and Guernsey breeds before sucking and for each day thereafter for one week. Plasma vitamin A and carotene determinations were made according to a previously published procedure (5) modified as suggested by Kimble (2). The calves were left with the cow for 3 days and thereafter received whole milk at the rate of about one pound for each 10 pounds of live weight.

Colostrum was withheld from 3 calves in order to study the effect on the vitamin A and carotene content of the blood plasma. Whole milk was fed in place of the colostrum. Three calves were permitted to receive colostrum for only 12 hours and three for 24 hours.

#### RESULTS AND DISCUSSION

The main results have been summarized in table 1 along with the standard error for each set of averages. The results show that the blood plasma of a new-born calf is low in vitamin A and carotene. A calf with similar low values at 3 to 12 months of age would show a marked deficiency and would not be able to survive. This might indicate a considerably greater requirement for vitamin A after birth than while being carried in utero. It could be argued that the calf carried in utero has no vitamin A requirement. This line of reasoning, however, is not valid because the same pathology is produced in utero if the dam is fed a deficient ration as is produced in vitamin A deficiency in a growing calf after birth.

After 24 hours the vitamin A content of the plasma increases four to five fold as would be expected because of the high vitamin A content of the colostrum. A maximum is usually reached at the third to fourth day after which there is a gradual decline. The decline is due to the fact that the calves are removed from the cow on the third day and fed whole milk which is lower in vitamin A. It seems likely, however, that the same decline would be noted even though the calves were left on the cow since by this time the secretion from the udder assumes the characteristics of whole milk. These data are in agreement with those reported by Lundquist and Phillips (4) in respect to the effect of colostrum on the vitamin A content of the blood plasma of new-born calves.

The data also show no particular breed variations in the vitamin A content of the blood plasma on comparable days. This would indicate that the



TABLE 1  
*Effect of colostrum on blood plasma vitamin A and carotene of calves for the first week*

Age in days	Holstein		Ayrshire		Jersey		Guernsey	
	No. of animals	$\mu\text{g}$ per 100 ml.	No. of animals	$\mu\text{g}$ per 100 ml.	No. of animals	$\mu\text{g}$ per 100 ml.	No. of animals	$\mu\text{g}$ per 100 ml.
Vitamin A								
0 (birth)	17	$3.3 \pm 0.48$	12	$3.6 \pm 0.57$	5	$2.4 \pm 0.45$	8	$4.2 \pm 0.84$
1	17	$15.6 \pm 1.80$	15	$13.2 \pm 1.32$	7	$14.1 \pm 2.07$	12	$16.5 \pm 1.38$
2	16	$16.8 \pm 1.34$	15	$17.1 \pm 1.14$	7	$20.1 \pm 2.43$	12	$16.4 \pm 1.64$
3	17	$15.9 \pm 1.32$	14	$18.3 \pm 0.93$	7	$17.4 \pm 2.10$	12	$15.6 \pm 1.32$
4	16	$15.0 \pm 1.44$	12	$15.0 \pm 0.78$	6	$15.9 \pm 1.44$	13	$14.1 \pm 1.29$
5	15	$14.4 \pm 1.47$	11	$16.2 \pm 1.59$	5	$14.1 \pm 1.05$	11	$15.0 \pm 0.96$
6	16	$13.2 \pm 1.26$	10	$14.7 \pm 1.38$	5	$9.9 \pm 1.11$	9	$15.0 \pm 0.48$
7	13	$13.8 \pm 0.84$	9	$15.3 \pm 1.02$	6	$11.4 \pm 0.84$	8	$15.9 \pm 1.56$
Carotene								
0 (birth)	17	$1.8 \pm 0.11$	12	$1.5 \pm 0.06$	5	$3.4 \pm 0.49$	8	$2.9 \pm 0.49$
1	17	$14.9 \pm 0.64$	15	$18.1 \pm 0.59$	7	$30.1 \pm 2.30$	12	$39.1 \pm 1.33$
2	16	$17.4 \pm 0.63$	15	$23.7 \pm 0.85$	7	$48.7 \pm 3.71$	12	$52.9 \pm 1.45$
3	17	$18.8 \pm 0.59$	14	$28.1 \pm 0.86$	7	$49.9 \pm 3.75$	12	$57.7 \pm 1.91$
4	16	$19.1 \pm 0.77$	12	$27.8 \pm 0.90$	6	$57.3 \pm 3.16$	13	$55.9 \pm 1.84$
5	15	$18.7 \pm 0.56$	11	$26.4 \pm 1.01$	5	$47.6 \pm 3.62$	11	$52.2 \pm 2.04$
6	16	$17.4 \pm 0.44$	11	$24.5 \pm 0.83$	5	$45.6 \pm 3.10$	10	$50.4 \pm 1.90$
7	13	$16.5 \pm 0.41$	9	$23.1 \pm 1.07$	6	$41.2 \pm 2.98$	8	$53.0 \pm 1.55$

supposedly greater difficulty of raising Jersey and Guernsey calves does not lie in the vitamin A metabolism during the first week of life.

The carotene content of the blood plasma of calves shows somewhat the same trend of increase as the vitamin A except here there are marked breed differences. The carotene is much higher for the Jersey and Guernsey breeds than for the Holstein and Ayrshire breeds. In this connection an Ayrshire calf 12 hours of age was placed on a Guernsey cow which had just freshened. The carotene values of the blood plasma of the Ayrshire calf on the Guernsey cow then assumed the high values noted for the Guernsey calves. This would indicate that the breed differences in the carotene content of the blood plasma of calves of this age was due to the carotene content of the colostrum of the breed and possibly not to differences in carotene metabolism in the calf as is true a little later in the growth period (6).

TABLE 2

*Vitamin A and carotene content of blood plasma of calves which did not respond to colostrum*

Age, days	Guernsey female*		Ayrshire female†		Holstein female‡	
	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	Carotene
<i>micrograms per 100 ml.</i>						
0	3.3	4.0	2.7	1.0	1.2	2.0
1	3.3	6.0	2.1	1.0	2.1	2.0
2	3.3	5.0	2.7	1.0	.....	.....
3	3.3	5.0	0.6	1.0	.....	.....
4	2.1	4.0	.....	.....	.....	.....
5	1.2	2.0	.....	.....	.....	.....
6	2.1	4.0	.....	.....	.....	.....
7	2.1	4.0	.....	.....	.....	.....

\* Died at 13 days of age.

† Died at 4 days of age.

‡ Died at 2 days of age.

It should be noted here that there were marked differences between calves of the same breed. These differences are probably related to the extreme variations previously noted in the vitamin A content of colostrum (1, 9, 12). These variations in colostrum might be of considerable practical importance since calves receiving colostrum of a low vitamin A value would not be able to build up their vitamin A reserve. Stewart and McCallum (12) noted that the longer the dry period the higher the vitamin A content of the colostrum. In three extreme cases the vitamin A and carotene values increased but little above the original values as shown in table 2. All of these calves died. Whether these observations were due to colostrum of poor quality or to an infection of the digestive tract which prevented absorption of vitamin A was not determined. It is possible that where the colostrum is low in vitamin A it might also be low in its content of immunizing bodies which the new-born calf needs in its defense mechanism against certain bacteria. This point would seem worthy of investigation.

TABLE 3

*Effect on blood plasma vitamin A and carotene of withholding colostrum*

Age, days	Holstein male		Holstein male*		Guernsey male†	
	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	Carotene
<i>micrograms per 100 ml.</i>						
0	2.7	2.0	0.6	1.0	3.6	4.0
1	3.0	3.0	1.8	1.0	3.9	4.0
2	3.3	4.0	1.5	3.0	.....	.....
3	3.9	6.0	3.9	5.0	.....	.....
4	6.0	8.0	1.8	4.0	.....	.....
5	4.6	6.0	1.2	9.0	.....	.....
6	4.5	8.0	3.0	7.0	.....	.....
7	6.9	10.0	2.7	9.0	.....	.....

\* Died 10 days of age.

† Sick at 2 days of age. Died at 3 days of age.

When three calves were not permitted to take colostrum but were fed whole milk instead, the blood plasma vitamin A and carotene values remained at a low level as shown in table 3. Two of these calves died, one at three days of age and one at ten days of age. This checks the observations of Smith and Little (10) who found that calves receive certain immune bodies from the colostrum to protect them against certain types of infection. One might be led to the conclusion that vitamin A also played some role in this regard since the vitamin A content of the blood plasma remains at a low level as shown in table 3. However, Smith and Little (11) also showed that the injection and ingestion of 200 to 300 ml. of cow serum prevented these early deaths when colostrum was withheld. It does not seem likely that the intake of this amount of serum would have any great effect on the level of blood plasma vitamin A or carotene. In studies with new-born calves the possible effect of these immunizing bodies on the health of the calf should not be overlooked in the interpretation of data related to nutrition.

The limited results from tables 4 and 5 indicate that the calf does not usually receive the full benefit from the colostrum in so far as vitamin A

TABLE 4

*Effect on blood plasma vitamin A and carotene of removing calf from cow after 12 hours*

Age, days	Guernsey male		Holstein male		Guernsey male	
	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	Carotene
<i>micrograms per 100 ml.</i>						
0	2.7	9.0	1.5	2.0	2.4	8.0
1	8.1	27.0	10.5	16.0	12.0	68.0
2	7.8	26.0	12.0	21.0	13.5	79.0
3	5.7	19.0	9.9	25.0	7.2	78.0
4	7.8	26.0	9.9	23.0	9.0	82.0
5	3.3	11.0	9.0	23.0	9.3	76.0
6	6.6	22.0	8.7	23.0	6.9	72.0
7	6.6	23.0	9.6	23.0	4.7	69.0

is concerned when removed from the dam at 12- and 24-hour periods after birth as indicated by the vitamin A values of the blood plasma when compared to the averages presented in table 1. The best practice would be to leave the calf with its dam for at least 3 days so as to take full advantage of the high vitamin A content of the colostrum unless of course the colostrum is hand-fed.

The intake of colostrum probably gives the new-born calf a chance to build up a considerable reserve of vitamin A in the liver. If the calf does not receive the colostrum, that reserve is not built up and may have considerable to do with the health of the calf during the first few months of life before hay is consumed to any great extent.

TABLE 5

*Effect on blood plasma vitamin A and carotene of removing calf from cow after 24 hours*

Age, days	Holstein male		Guernsey male		Holstein male	
	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	Carotene
<i>micrograms per 100 ml.</i>						
0	3.6	2.0	2.1	4.0	.....	1.0
1	19.5	18.0	6.8	23.0	13.5	3.0
2	22.5	22.0	7.5	43.0	10.8	6.0
3	15.0	22.0	.....	.....	12.0	10.0
4	9.5	19.0	6.6	37.0	9.3	14.0
5	13.5	15.0	7.0	39.0	10.5	10.0
6	15.9	15.0	7.5	38.0	7.2	8.0
7	7.7	15.0	9.9	38.0	12.0	9.0

The colostrum milk on most dairy farms is discarded since it cannot be included in milk sold for market purposes. This would appear to be most wasteful of a large amount of vitamin A and many other factors necessary for the health of calves. Studies should be made to determine whether this milk could be mixed with the whole milk fed with some possible benefit to the calves being raised in the herd.

#### SUMMARY

1. The vitamin A content of the blood plasma of dairy calves at birth was low but at the end of 24 hours usually showed about a 5-fold increase with the intake of colostrum.

2. Maximal vitamin A and carotene values were attained at about three days of age after which there was a gradual decline.

3. There was no marked difference between breeds in the vitamin A content of the blood plasma at comparable ages; however, the carotene content of the blood plasma of the Jersey and Guernsey breeds was three to four times higher than for the Holstein breed.

4. In three cases neither the plasma vitamin A nor carotene values showed an increase even though the calves apparently consumed the colostrum. These calves died from infection.

5. In three cases where the colostrum was withheld and whole milk was fed, the plasma vitamin A and carotene content showed very little increase. Two of these calves died of infection.

6. The plasma vitamin A and carotene values did not reach the maximum values where the calves were removed at 12 and 24 hours after birth.

7. The intake of colostrum probably gives the calf a chance to build up a considerable reserve of vitamin A in the liver. If the calf does not receive the colostrum, that reserve is not built up and may have considerable to do with the health of the calf during the first two months.

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# THE EFFECT OF STORAGE ON THE RETENTION OF CERTAIN MEMBERS OF THE VITAMIN B COMPLEX\*

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Considerable work has been done on the stability of carotene under storage on such plant products as alfalfa, clover, timothy hays, alfalfa leaf meal, etc. With the ever-increasing knowledge of the role of the B complex

TABLE 1  
*Thiamine*

No.	Material	New material, fall—1942	One year storage, ground	One year storage, unground
<i>milligrams per 100 grams</i>				
1	Alfalfa hay	0.23	0.23	0.23
2	Timothy hay	0.17	0.19	0.17
3	Clover hay	0.14	0.19	0.18
4	Alfalfa leaf meal	0.26	0.21	0.13
5	Cerophyll	0.31	0.41	0.38
6	Peas	0.23	0.23	0.23
7	Pea pods	0.24	0.24	0.25
8	Soybeans	1.55	1.56	1.47
9	Yellow corn	0.59	0.54	0.55
10	White corn	0.43	0.45	0.42
11	Navy beans	0.47	0.54	0.54
12	Oats—Vieland	0.57	0.47	0.46
13	Oats—Ped. No. 7	0.43	0.77	0.42
14	Oats—X219-1*	0.38	0.39	0.38
15	Oats—CI 3662	0.26	0.26	0.26
16	Oats—Erban	0.38	0.39	0.36
17	Barley—Ped. 38	0.61	0.44	0.48
18	Barley—Ped. 5-1	0.56	0.59	0.53
19	Barley—X191-2-1-2	0.62	0.66	0.65
20	Rye—Imperial	0.19	0.22	0.22
21	Rye—Dakold	0.23	0.27	0.27
22	Rye—No. 7	0.24	0.25	0.22
23	S.W.†—Pilot 13	0.37	0.45	0.39
24	S.W.—H157A-4-12-8	0.36	0.32	0.26
25	S.W.—Thatcher	0.37	0.38	0.35
26	S.W.—Sturgeon	0.32	0.28	0.26
27	W.W.‡—H59-13-9-5-7-12	0.17	0.18	0.16
28	W.W.—Minturki	0.12	0.14	0.15
29	W.W.— $\frac{312.27}{29.13}$	0.22	0.26	0.24

\* The products that are followed by serial numbers are experimental varieties grown by the department of agronomy.

† S.W.—Spring wheat.

‡ W.W.—Winter wheat.

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in nutrition, work should be carried out on the effect of storage on the retention of these essential nutrients.

Experiments conducted at the Iowa State College indicate that the thiamine content of ideally-stored, whole yellow corn (3) does not appear to be affected by aging even as long as four years. Studies on rice also have indicated that this vitamin is quite stable during storage. Hulled rice (4)

TABLE 2

*Riboflavin*

No.	Material	New material, fall—1942	One year storage, ground	One year storage, unground
<i>milligrams per 100 grams</i>				
1	Alfalfa hay	0.56	0.43	0.44
2	Timothy hay	0.54	0.56	0.51
3	Clover hay	0.69	0.56	0.53
4	Alfalfa leaf meal	1.67	1.61	1.62
5	Cerophyll	1.67	1.71	1.70
6	Peas	0.39	0.42	0.39
7	Pea pods	0.63	0.71	0.64
8	Soybeans	0.35	0.30	0.37
9	Yellow corn	0.16	0.18	0.17
10	White corn	0.15	0.14	0.13
11	Navy beans	0.14	0.14	0.15
12	Oats—Vicland	0.11	0.14	0.13
13	Oats—Ped. No. 7	0.33	0.30	0.32
14	Oats—X219-1*	0.21	0.26	0.27
15	Oats—CI 3662	0.16	0.20	0.14
16	Oats—Erban	0.19	0.23	0.16
17	Barley—Ped. 38	0.15	0.15	0.14
18	Barley—Ped. 5-1	0.22	0.29	0.20
19	Barley—X191-2-1-2	0.26	0.27	0.29
20	Rye—Imperial	0.18	0.13	0.21
21	Rye—Dakold	0.28	0.25	0.23
22	Rye—No. 7	0.28	0.26	0.26
23	S.W.†—Pilot 13	0.18	0.18	0.25
24	S.W.—H157A-4-12-8	0.21	0.24	0.24
25	S.W.—Thatcher	0.17	0.17	0.20
26	S.W.—Sturgeon	0.18	0.18	0.20
27	W.W.‡—H59-13-9-5-7-12	0.18	0.15	0.20
28	W.W.—Minturki	0.13	0.15	0.20
29	W.W.— $\frac{312.27}{29.13}$	0.17	0.15	0.17

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stored in straw bags for four years retained most of its original thiamine content for the first two years, with a significant drop at the end of the third and fourth year. After storage in air-tight containers for 26–28 years, hulled rice (5) still retained 84 and 54 per cent of its original thiamine content. Hulled rice (6) stored in hermetically sealed concrete silos for five years showed no appreciable depreciation in thiamine content. A moisture content of rice (7) greater than 10 per cent causes a decrease in the thiamine



content, and the loss of vitamin runs parallel with that of viability of the seed.

Riboflavin seems to be quite stable. Fraps (3) reported that some alfalfa leaf meal which had been stored so long that almost all of the carotene had disappeared still had the same riboflavin content as is usually found in alfalfa leaf meal.

TABLE 3  
*Nicotinic acid*

No.	Material	New material, fall—1942	One year storage, ground	One year storage, unground
<i>milligrams per 100 grams</i>				
1	Alfalfa hay	2.85	2.75	2.70
2	Timothy hay	2.35	2.00	1.94
3	Clover hay	2.85	2.95	2.70
4	Alfalfa leaf meal	4.55	4.30	3.98
5	Cerophyll	4.55	4.76	4.84
6	Peas	3.70	3.75	3.41
7	Pea pods	2.75	2.44	2.70
8	Soybeans	2.12	1.80	1.84
9	Yellow corn	2.32	2.45	2.38
10	White corn	2.36	2.52	2.38
11	Navy beans	1.75	2.07	2.15
12	Oats—Vicland	0.73	0.76	0.73
13	Oats—Ped. No. 7	0.75	0.84	0.78
14	Oats—X219-1*	0.65	0.78	0.68
15	Oats—CI 3662	0.64	0.68	0.66
16	Oats—Erban	0.59	0.69	0.71
17	Barley—Ped. 38	4.45	4.44	4.69
18	Barley—Ped. 5-1	4.46	4.44	4.58
19	Barley—X191-2-1-2	4.92	4.89	5.07
20	Rye—Imperial	0.68	0.69	0.76
21	Rye—Dakold	1.27	1.03	1.00
22	Rye—No. 7	1.25	0.75	1.05
23	S.W.†—Pilot 13	6.52	6.73	6.54
24	S.W.—H157A-4-12-8	4.52	5.19	4.90
25	S.W.—Thatcher	6.28	5.99	5.64
26	S.W.—Sturgeon	5.36	5.76	5.53
27	W.W.‡—H59-13-9-5-7-12	4.36	4.26	4.77
28	W.W.—Minturki	5.06	4.40	4.43
29	W.W.— $\frac{312.27}{29.13}$	4.72	4.53	4.38

\* The products that are followed by serial numbers are experimental varieties grown by the department of agronomy.

† S.W.—Spring wheat.

‡ W.W.—Winter wheat.

Douglass and Richardson (1) in 1930, found that the vitamin B (complex) in carrots was not affected upon storage. In a later paper by Richardson, Langely and Andes (9) the same results were obtained.

It is obvious from the above data that very little work has been done on this particular problem. With this in mind, the following experiments were carried out. Twenty-nine air-dried plant products were obtained from the 1942 crop and assayed for riboflavin, thiamine, biotin, nicotinic acid and

pantothenic acid. The remainder of the sample was divided into two parts; part one was ground in a small feed grinder and stored in paper bags; part two was unground but stored under similar conditions. After six months storage, at room temperature, 20–25° C., the samples were reassayed for the above members of the B complex. This process was repeated after one year

TABLE 4  
*Pantothenic acid*

No.	Material	New material, fall—1942	One year storage, ground	One year storage, unground
<i>milligrams per 100 grams</i>				
1	Alfalfa hay	1.58	1.48	1.49
2	Timothy hay	0.85	0.65	0.60
3	Clover hay	1.00	0.87	0.80
4	Alfalfa leaf meal	2.50	2.30	2.27
5	Cerophyll	0.69	0.71	0.74
6	Peas	1.01	0.99	0.98
7	Pea pods	0.99	0.95	0.95
8	Soybeans	1.24	1.01	0.96
9	Yellow corn	0.48	0.42	0.42
10	White corn	0.48	0.42	0.42
11	Navy beans	0.46	0.40	0.37
12	Oats—Vieland	0.76	0.54	0.52
13	Oats—Ped. No. 7	0.99	0.64	0.64
14	Oats—X219-1*	0.65	0.56	0.56
15	Oats—CI 3662	0.54	0.44	0.48
16	Oats—Erban	0.58	0.60	0.63
17	Barley—Ped. 38	0.32	0.32	0.34
18	Barley—Ped. 5-1	0.35	0.29	0.28
19	Barley—X191-2-1-2	0.27	0.30	0.25
20	Rye—Imperial	0.28	0.26	0.28
21	Rye—Dakold	0.23	0.26	0.25
22	Rye—No. 7	0.22	0.33	0.30
23	S.W.†—Pilot 13	1.33	1.35	1.37
24	S.W.—H157A-4-12-8	1.50	1.46	1.47
25	S.W.—Thatcher	1.28	1.56	1.49
26	S.W.—Sturgeon	1.23	1.27	1.19
27	W.W.‡—H59-13-9-5-7-12	1.20	1.20	1.26
28	W.W.—Minturki	1.25	1.32	1.21
29	W.W.— 312.27 29.13	1.17	1.22	1.33

\* The products that are followed by serial numbers are experimental varieties grown by the department of agronomy.

† S.W.—Spring wheat.

‡ W.W.—Winter wheat.

of storage. Only the data on the effect of storage for one year are reported in this paper. At six months storage no change had taken place.

#### EXPERIMENTAL

The samples were first prepared for assaying by grinding in a Wiley mill. Difficulties were encountered in grinding such seed products as wheat, rye and oats. It was practically impossible to grind the outer coatings so the only part of the sample that was assayed was that portion which came

through a 40-mesh sieve. Using the above procedure the results were very low, particularly in relation to nicotinic acid and pantothenic acid. It was found that if the samples were ground in a small feed grinder, without sieving, the results checked very well with previous reports. All of the subsequent samples were prepared by grinding in the small feed mill.

TABLE 5

*Biotin*

No.	Material	New material, fall—1942	One year storage, ground	One year storage, unground
<i>millimicrograms per gram</i>				
1	Alfalfa hay	127.8	179.0	174.0
2	Timothy hay	32.0	65.7	66.7
3	Clover hay	77.0	94.0	89.0
4	Alfalfa leaf meal	245.0	330.0	347.0
5	Cerophyll	155.0	285.0	269.0
6	Peas	224.0	205.0	209.0
7	Pea pods	241.0	219.0	228.0
8	Soybeans	356.0	385.0	373.0
9	Yellow corn	54.6	77.0	85.0
10	White corn	73.0	80.0	80.0
11	Navy beans	76.3	120.0	112.0
12	Oats—Vieland	207.0	251.0	269.0
13	Oats—Ped. No. 7	141.3	287.0	296.0
14	Oats—X219-1*	175.0	281.0	320.0
15	Oats—CI 3662	158.7	294.0	331.0
16	Oats—Erban	319.0	301.0	231.0
17	Barley—Ped. 38	79.6	231.0	231.0
18	Barley—Ped. 5-1	106.3	97.0	97.0
19	Barley—X191-2-1-2	111.0	81.0	101.0
20	Rye—Imperial	59.8	77.4	83.9
21	Rye—Dakold	61.1	70.0	74.2
22	Rye—No. 7	65.1	60.0	63.0
23	S.W.†—Pilot 13	69.6	80.8	83.9
24	S.W.—H157A-4-12-8	44.0	140.0	120.0
25	S.W.—Thatcher	25.6	81.0	83.2
26	S.W.—Sturgeon	36.6	130.0	132.0
27	W.W.‡—H59-13-9-5-7-12	65.0	80.0	92.0
28	W.W.—Minturki	59.5	70.6	83.0
29	W.W.— $\frac{312.27}{29.13}$	68.9	73.0	64.0

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† S.W.—Spring wheat.

‡ W.W.—Winter wheat.

Each sample was assayed for the following members of the B complex according to the accepted procedures: riboflavin (12-14), pantothenic acid (15), thiamine (2), biotin (10-11), and nicotinic acid (8-13).

## RESULTS

It is evident from the tables that no losses are encountered in these five members of the B complex upon storage. Of course, the year period of storage may not have been long enough, but we feel quite sure that under natural storing conditions these members of the B complex are stable.

It should be observed from the tables that in the case of biotin the values are higher after storage than in the original product. This can be explained by the fact that the original medium used for the assay was not complete. A factor obtained from yeast, essential for the growth of the bacteria, was not supplied in sufficient amounts. The latter analyses were made under the modified method (11).

#### SUMMARY

Twenty-nine samples of plant products including hays and grains were assayed for thiamine, riboflavin, biotin, nicotinic acid and pantothenic acid. These samples were stored in the dark at 20–25° C. for one year, ground and unground, with no apparent loss of the vitamins mentioned above.

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## TESTING FOR EXTRANEEOUS MATTER IN CHEESE\*

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The cheese sediment test is intended to detect cheese made from unclean milk or cheese made under unsanitary conditions or both; and, second, to demonstrate to milk and cheese producers the necessity of strict cleanliness. There is a limiting factor which may interfere with the effectiveness of the test and may actually make it a deceiving instrument. When the milk is efficiently filtered or clarified in the factory then a clean cheese sediment test is not necessarily indicative of a satisfactory milk supply.

It is general practice in the industry to inspect cheese sediment tests and to classify them according to certain arbitrary standards. The grades generally used are Good, Fair, Poor and Illegal and are numbered 1, 2, 3 and 4, respectively, to correspond, presumably, with the four grades of milk sediment tests commonly recognized in milk manufacturing areas.<sup>1</sup> Milk sediment tests are classified only by amounts of sediment visible to the unaided eye but cheese sediment tests are also examined microscopically for material of insect or animal origin before the tests can be properly classified.

### METHODS OF MAKING TESTS FOR EXTRANEEOUS MATTER IN CHEESE

Two procedures are commonly used for testing cheese for extraneous matter; one test requires sodium citrate (2) as a solvent while the other employs orthophosphoric acid (3). A third test was developed by Dr. Chernoff in the Denver Station of the Food and Drug Administration (1); it has not found application in commercial practice. A modification of the sodium citrate test is probably most commonly followed at present.

It is our purpose to discuss the commonly used tests and some of the problems of interpreting and applying them in commercial practice. In table 1 are shown the essential characteristics of the phosphoric acid test and the original and modified forms of the sodium citrate tests.

The sediment discs from the test are graded macroscopically and are examined with a low-power microscope to detect the presence of critical foreign matter of animal or insect origin.

It is impossible to identify or even to discover some types of critical extraneous material, such as fine hairs, for example, without some magnification. This fact is illustrated by a series of 1,102 extraneous matter tests.

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<sup>1</sup> The State of Illinois, Division of Foods and Dairies, 228 South Wabash Avenue, Chicago, Illinois, published a pamphlet in 1942 which illustrates both milk sediment and cheese sediment standards of this type.

The tests were first examined carefully with the naked eye and 9 of them were given a grade of 4, a grade which is an indication of excessive quantities of extraneous material or the presence of critical material such as rodent hair, cow hair or insect parts. When the same 1,102 tests were examined with 85 $\times$  magnification 481 sediment pads were graded 4. Visual inspection of cheese sediment tests must always be supplemented with microscopic examination if the test is to serve its fullest usefulness in identifying and correcting sources of contamination.

TABLE 1  
*Methods of detecting extraneous matter in cheese*

	Methods		
	Original sodium citrate	Modified sodium citrate	Phosphoric acid
1. Size of sample .....	100 gm.	225 gm. (8 oz.)	50 gm.
2. Type of solvent ...	Sodium citrate	Sodium citrate	Orthophosphoric acid
3. Strength of solvent .....	150 gm. in 1000 ml. of water	100 gm. in 1000 ml. of water	7 ml. in 1000 ml. of water
4. Amount of solvent .....	300 ml.	800 ml.	500 ml.
5. Solvent contact before heating .....	None	Overnight	None
6. Temperature of heating .....	140° F.	150° F.	Boiling
7. Duration of heating .....	About 30 min.	About 45 min.	Until dissolved
8. Agitation .....	Vigorous	Vigorous	Occasional
9. Filter disc .....	Reversed 1½" milk-sediment disc		1½" Irish poplin disc*
10. Filtering surface of disc .....	One-inch circle	One-inch circle	One-inch circle
11. Filtering device ...	Pressure or vacuumatic milk sediment tester		

\* The Irish poplin is used over a milk sediment pad.

#### USING THE MICROSCOPE TO ESTIMATE THE AMOUNT OF EXTRANEOUS MATTER ON SEDIMENT DISCS

The idea of grading sediment pads by numbers of particles of extraneous matter was investigated. For counting particles of extraneous matter a low-power, binocular microscope was used; its three objectives provided magnifications of 21.2, 42.5 and 85 diameters. A jig for holding a sediment pad was placed in the clamps of the mechanical stage so that it was possible to examine the entire surface of the pad systematically in a minimum of time.

The actual counting operation was facilitated by the use of a reticule in the eyepiece of the binocular that was ruled into squares and served to mark off the boundaries of a definite area of the microscopic field; this area was measured. The known area of the filtering surface of the sediment pad

divided by the measured area in the microscopic field gave a factor which, when multiplied by the average count per field, gave an estimate of the number of particles on the sediment pad. The average count of particles per field was based upon the counting of approximately 20 fields.

Experiments, which need not be described in detail, showed that macroscopic grades are related to numbers of particles but the labor involved does not justify microscopic counting for commercial grading. The method was very useful in some phases of this study.

#### FILTERING MATERIALS FOR EXTRANEOUS MATTER TESTS

Careful microscopic examination of the reversed, milk-sediment discs indicated that short segments of hairs and fine particles of extraneous matter may become so deeply imbedded even in the compact side of the discs that undoubtedly some must escape detection altogether. Irish poplin was found

TABLE 2

*Comparison of the retaining power of the reversed milk sediment disc and Irish poplin disc*

Sample number	Solvent	Number of particles from 50 grams of cheese		Ratio of counts on the two types of sediment discs
		Irish poplin disc	Milk sediment disc	
1	Acid	7812	1220	(poplin : milk disc) 6 : 1
2	Acid	18648	1465	13 : 1
3	Acid	16632	1720	10 : 1
4	Citrate	6552	722	9 : 1
5	Citrate	16632	2050	8 : 1
6	Citrate	14868	2210	7 : 1
7	Citrate	16632	2440	7 : 1

to be more satisfactory when used over the usual milk sediment disc. This closely woven cloth provides a relatively smooth surface on which extraneous matter can be examined microscopically with a minimum of focusing. One disc of Irish poplin clogs before eight ounces of dissolved cheese can be filtered through it but with a 50-gram sample it rarely if ever gives any trouble.

A measure of the hiding effect of the milk sediment disc is indicated in table 2 which shows the number of visible particles of sediment appearing on both types of filters when samples of the same size from identical cheese are tested. In these trials the poplin discs revealed 6 to 13 times as many particles under  $85\times$  magnification as the reversed milk sediment discs. This ratio tends to vary with the type and size of extraneous matter particles in the cheese. When the Irish poplin pad is used it has the practical effect of making the results of the test with the 50-gram sample of cheese equivalent to a larger sample filtered through a reversed milk sediment disc.

## EFFECTS OF SOLVENTS ON EXTRANEOUS MATTER

Spicer and Price (2) observed no marked effect on common types of extraneous material surviving their test nor after treating the cheese with the citrate solvent for 18 hours at 37° C. This type of soaking period is now commonly used in commercial laboratories to hasten the period of solution when the sample is placed in the heating bath. Turner, Rogers and Conquest (3) stated that cellulose, chitin and inorganic types of extraneous matter lost approximately 5 per cent of their weight during 20 minutes of boiling in the one per cent orthophosphoric acid solution, and furthermore, that their structures were not visibly altered.

TABLE 3

*The influence of type of solvent on extraneous matter*  
(Sodium citrate, 10% solution; orthophosphoric acid, 1% solution)

Type of extraneous material*	Solvent	Amount of solvent	Maximum temperature	Duration of heating	Proportion dissolved
		<i>ml.</i>	<i>° F.</i>	<i>min.</i>	<i>%</i>
Milk sediment	Citrate	200	150	15	33
	Acid	500	212	15	51
	Water	160	212	15	16
Cow dung	Citrate	200	150	15	20
	Acid	500	212	15	22
Timothy hay	Citrate	200	150	15	7
	Acid	500	212	15	18
Oat hulls	Citrate	200	150	15	20
	Acid	500	212	15	30
Hay and grain mixture	Citrate	800	150	30	21
	Acid	500	212	15	27
Iron filings	Citrate	200	150	15	6
	Acid	500	212	15	100

\* Extraneous material was dried at 100° F. before weighing both before and after solvent action.

During this study such materials as hair, dust, insect parts, brush bristles, spider web, wood splinters, metal, grease from an agitator, and cotton threads from bandages have been subjected to the conditions and solvents of each test. These materials in general have been recovered after the treatments in identifiable amounts with the exception of cotton bandage threads which were dissolved by the sodium citrate solvent; agitator grease, which was dissolved by both solvents and iron particles which were dissolved by the acid. Both solvents were found to have some dissolving action on common materials such as those shown in table 3. The substances shown in this table were subjected to treatments approximating those of the actual tests; no cheese was present in the solutions during the treatments.

Table 3 indicates that the phosphoric acid solvent always tends to dissolve a little more of the organic matter than the citrate and attacks iron



strongly. Despite the amounts of the substances dissolved by either test there still remained on the filter pads enough of the substances for identification and observation. It is interesting to observe that even boiling water has a marked action on samples of dried milk-sediment taken from a factory filter cloth.

The conditions of the treatments shown in table 3 are probably more severe than those to which these substances would ordinarily be exposed in the extraneous matter test. In making the test the cheese itself acts as a protecting cover for at least a portion of the extraneous matter while the cheese is being dissolved; then, as soon as possible after the cheese is dispersed, the solution is filtered.

Sodium citrate is distinctly less active toward metals than the phosphoric acid. This fact is important in the choice of materials used for handling the cheese and phosphoric acid solvents while testing for extraneous matter. A poorly tinned or copper sediment tester may contribute so much dissolved metal to the acid solution that the sediment pad may be practically worthless as an indication of the condition of the cheese.

#### THE INFLUENCE OF CONTINUED HEATING UPON EXTRANEEOUS MATERIAL

There is a possibility that the extraneous matter in cheese might dissolve if the duration of the heat treatment were increased as is sometimes neces-

TABLE 4

*The influence of the duration of the heat treatment on the extraneous matter*

Duration of heating	Average number of particles on poplin pads	
	Sodium citrate solvent	Phosphoric acid solvent
0.5 hour .....	7,790	11,220
1.0 hour .....	8,970	12,200
1.5 hours .....	7,380	11,680
2.0 hours .....	8,010	11,170

sary to obtain complete dispersion. This question was studied by grinding up several pounds of cheese, dividing it into several portions and finally subjecting these portions to four different heat treatments with each of the two solvents. During the treatment with sodium citrate the cheese-solvent mixture was stirred gently while being held at the usual 150° F.; with phosphoric acid the slow boiling of the mixture furnished the only agitation. All mixtures were filtered through Irish poplin and the particles of extraneous matter were counted under 85× magnification. The results are shown in table 4.

The data in table 4 show that prolonging the heat treatments does not apparently change the results of the tests. If there is a tendency for the continued action of the solvents to dissolve the extraneous matter it is not

revealed by counting the number of particles remaining on the filter discs; neither is it apparent to the naked eye in the discoloration of the filter discs.

The data in table 4 also show a distinctly larger number of particles revealed by the phosphoric acid treatment. This suggested the necessity of further observations of the effect of solvent on numbers and size of sediment particles.

INFLUENCE OF SOLVENTS UPON THE SIZE AND NUMBER OF PARTICLES OF SEDIMENT REMAINING ON THE SEDIMENT PADS AFTER FILTERING

Occasional sediment tests with sodium citrate seem darker than tests made with phosphoric acid on identical samples of cheese. Such effects might be caused by differences in the size or number of particles surviving the test treatments.

TABLE 5  
*Influence of solvents on size of particles of sediment*

Cheese sample number	Average diameter of particles in 10 random fields					
	50-gram samples on poplin			8-oz. samples on milk filter		
	Sodium citrate	Phosphoric acid	Difference (Citrate - Acid)	Sodium citrate	Phosphoric acid	Difference (Citrate - Acid)
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1	0.074	0.060	0.014	0.096	0.050	0.046
2	0.066	0.045	0.021	0.088	0.068	0.020
3	0.060	0.038	0.022	0.088	0.097	-0.009
4	0.060	0.036	0.024	0.084	0.092	-0.008
5	0.058	0.048	0.010	0.062	0.058	0.004
6	0.054	0.044	0.010	0.086	0.084	0.002
7	0.072	0.033	0.039	.....	.....	.....
Average	0.063	0.043	0.020	0.084	0.0748	0.009

*Size.* Measurements were made of size of particles by using both sediment tests on identical samples of cheese. Some of the tests were 50-gram samples filtered through poplin discs, others were 8-ounce samples filtered through milk sediment discs. Each disc was placed under the microscope and the longest dimension of the particle nearest the center of each field was recorded. Ten fields on each disc were selected at random and particles were so measured; the average of these measurements is shown in table 5.

It is apparent that when the poplin filter pad is used the particles resulting from the phosphoric acid treatment are about two-thirds the size of those remaining after the sodium citrate treatment. When the milk filter disc is used the average size of particles is larger and practically the same for both solvents. Here again the hiding effect of the milk sediment disc is indicated.

*Number.* In table 6 are shown numbers of particles from equal amounts of identical cheese tested with each solvent. Samples 1 to 8 in table 6 were filtered on Irish poplin; the citrate test showed approximately 1 particle to

each 1.8 particles from the test with acid; when the reversed, milk-sediment, filter disc was used for samples 9 to 13 then the ratio of counts averaged practically 1 to 0.9.

Such differences in the ratio of numbers of particles resulting when the two solvents are used can be explained by the tendency of the acid to break up and so reduce the size of particles. The poplin filter disc retains the finer particles on its surface so that the number visible is distinctly greater than that resulting when the milk sediment disc is used. On the other hand, the finer particles penetrate the loose structure of the milk sediment disc leaving a proportionately greater number of large particles visible. On the

TABLE 6

*The influence of the solvent on the number of particles of extraneous matter*

Sample number	Number of particles from 50 gms. of cheese		Ratio of counts (citrate : acid)
	Sodium citrate	Phosphoric acid	
	Irish poplin filter disc		
1	16,130	29,480	1 : 1.8
2	12,850	27,720	1 : 2.2
3	17,890	24,440	1 : 1.4
4	12,820	19,150	1 : 1.5
5	14,870	18,650	1 : 1.3
6	16,630	28,980	1 : 1.7
7	7,310	17,640	1 : 2.4
8	7,060	15,120	1 : 2.1
	Reversed milk sediment disc		
9	2,440	1,720	1 : 0.7
10	2,220	1,460	1 : 0.7
11	2,420	1,550	1 : 0.6
12	1,250	1,140	1 : 0.9
13	2,250	3,276	1 : 1.5

Irish poplin the acid test may show the darker appearance depending somewhat on the type and color of the finely divided extraneous matter present in the cheese but on the reversed milk-sediment pad the citrate test is apt to show the darker color.

#### ESTIMATING SEDIMENT GRADES BY MACROSCOPIC EXAMINATION

Three judges were asked to grade unidentified sediment test discs drawn at random from 237 obtained by analyzing three samples from each of 79 cheddars making up 8 different lots of cheese. The summary of their estimates is presented in table 7 which shows the macroscopic grade for each lot determined by the majority of each judge's votes and the proportion of his votes disagreeing with his majority. The proportion of "maverick" votes indicates the variability of each lot in the opinion of that judge.

The judges did not agree either in their estimates of the uniformity of the samples from each lot of cheese or on the grades of the lots themselves. It is obvious that variations in samples from lots as well as variations in judges' opinions are involved. The judges indicated that unanimous agreement would be impossible when grading unidentified sediment test discs; one reason was the "border-line" samples which might be called one grade one time and another grade at another inspection; another reason was the personal factor which included speed of examination, eyesight, leniency, distractions while judging, light conditions, and finally the experience of the judge. Experience in judging discs however does not contribute necessarily to the uniformity of the results because Judge A in table 7 was by far the most experienced of the three.

TABLE 7

*Variability of judges in estimating grades by macroscopic examination*

	Size of test sample							
	50-gm. sample					8-oz. sample		
Lot number .....	1	2	3	4	5	6	7	8
Number of cheddars .....	4	12	10	10	9	10	12	12
Discs judged .....	12	33	30	30	27	30	36	36
Judge	Sediment grades indicated by majority votes							
A .....	2	2	2	3	3	4	2	2
B .....	3	3	2	3	4	4	2	2
C .....	3	2	2	3	3	4	2	2
Judge	Proportion of votes in the minority							
	%	%	%	%	%	%	%	%
A .....	25.0	8.3	23.3	23.3	51.8*	43.3	13.9	8.3
B .....	16.7	41.7	10.0	20.0	37.0	26.6	0.0	2.8
C .....	0.0	45.5	6.7	11.1	48.2	43.3	19.4	25.0

\* Over half of votes can be in the minority when all votes are distributed between more than two grades.

#### SAMPLING

The sample of cheese for sediment test must be removed from the lot in such amounts and in such a manner that it is typical of the whole lot. The removal of such a sample depends upon the uniformity of distribution of sediment between the individual cheeses constituting the lot and within each individual cheese.

*Distribution of sediment within the individual cheese.* The distribution of sediment within the individual cheese was studied by taking samples from three portions of each cheddar of eight different lots of cheese—a total of 79

cheddars. Each cheddar was sampled in three zones—top, bottom and center. Samples were obtained from the top and bottom by inserting a cheese trier obliquely into the flat surface at points midway between the center and circumference; the center sample was taken by inserting a butter trier straight down through the axis of the cheddar and removing the necessary amount from that portion of the plug nearest the center of the cheese. Fifty-gram samples were taken from 5 lots of 48 cheddars; they were tested by the phosphoric acid method, filtered through poplin and then mounted on sediment test cards under cellophane. Eight-ounce samples were taken from 3 lots of 31 cheddars; they were tested by the sodium citrate method, filtered on reversed milk sediment discs and then mounted on cards under cellophane. Judges were required to mix all cards from all cheddars into one pile from which tests were drawn and classified before any identifying marks were disclosed.

TABLE 8

*The distribution of sediment discs from 3 sections of cheddars by visual grading of three judges*

Judge	Number of cheddars	Number of discs judged	Minority votes		Distribution of minority vote		
			Number	Per cent	Top	Center	Bottom
A .....	79	237	55	23	20	22	13
B .....	79	237	45	19	10	20	15
C .....	77	231	62	27	19	25	18
Totals .....	235	705	162	23	49	67	46

The results of the classification of sediment tests are shown in table 8. The cheddars of each lot were regarded as a group and all sediment tests on that lot were used to determine the judge's grade of the lot. The grade of the lot was fixed by the majority of the judge's estimates of all tests on the lot. The estimates of grades in the minority ("mavericks"), are divided in table 8 according to the section from which the test was obtained.

Theoretically, if the extraneous matter were distributed evenly then the number of "mavericks" should be the same in each class. Out of a total of 705 sediment discs classified by the three judges, 162 were "mavericks." Of this 162, 49 samples were taken from the top, 67 from the center and 46 from the bottom of the cheddars. According to the chi-square test, this distribution is unusual but not significantly different from the equal number which was expected in each section. When the votes of the individual judges are considered by the same chi-square test it is clear that, as individuals, they found no single section producing significantly more "mavericks" than any other section.

Another attempt was made to discover, if possible, any tendency for the extraneous matter to occur in one of the three sections of these lots of cheese under discussion. This was done by estimating, under  $85\times$  magnification,

TABLE 9

*Number of particles on poplin sediment discs from tests of three sections of cheddars*

Section of cheese	Number of cheese	Numbers of sediment particles			
		Minimum	Maximum	Average	Standard deviation
Top .....	48	13,104	41,832	27,292	7,350
Center .....	48	14,112	45,864	26,875	7,250
Bottom .....	48	11,844	45,612	28,645	8,185

the number of particles of sediment on Irish poplin sediment discs obtained from five lots, totaling 48 cheddars. The results are shown in table 9. Typical data for all cheese in a single lot are shown in table 11.

The average number of particles of sediment on the discs from each of the three sections of the cheddars are practically identical, according to evidence in table 9. The ranges of average numbers of particles observed in each section are very similar. The variability of the data is also indicated by the magnitude of the standard deviations.

For all practical purposes it makes no difference in testing cheddars for extraneous matter whether the samples are taken from the top, center or bottom sections.

*Distribution of sediment between cheese in the lot.* The distribution of sediment between cheese constituting individual lots (vats) was not uniform as determined by microscopic counts of particles. Results of counts are summarized in table 10.

It has been shown that the distribution between sections of a cheese is uniform so that data from the top section of the cheese are used in table 10 to illustrate the variations between cheese. Median values in lots 1, 2 and 4 are practically identical; the range of counts indicated by minimum and maximum values in these three lots include the median values of the other two lots, 3 and 5, and almost include the extremes as well.

As further evidence of this lack of uniformity of distribution of extraneous matter, table 11 is presented. In this table is shown the number of particles of sediment in the top, center and bottom sections of the 12 ched-

TABLE 10

*Distribution of extraneous matter between cheese in the lot*

Lot number	Number of cheese	Number of particles in top section (50-gm. sample)		
		Minimum	Maximum	Median
1	12	20,160	38,050	25,830
2	8	19,908	31,248	25,956
3	10	13,104	20,916	17,514
4	10	21,420	34,776	25,964
5	9	27,468	43,344	33,516

dars constituting one lot or vat of cheese. Variations in counts in the three sections within individual cheese approximate 3500 particles; between individual cheese the range is from 19,000 to 39,000.

TABLE 11  
*Distribution of sediment in a typical lot of cheese*

Cheese number	Number of particles in samples (000 omitted)		
	Top	Center	Bottom
1	35	33	30
2	34	33	33
3	38	39	39
4	35	37	36
5	25	32	30
6	26	34	32
7	25	24	31
8	23	25	24
9	20	21	21
10	20	19	24
11	27	27	25
12	25	23	31

Macroscopic examination of sediment tests from every cheese in a single lot revealed definite differences in amounts of extraneous matter. In table 12 the ratings of one experienced judge are used. All sediment discs were made from samples from the top sections of each cheese in each lot.

The sediment tests on these 50-gram samples of different cheese in the same lot indicate that more extraneous matter can be expected in some cheese than in others. Two lots in table 12 show discs in three of the four possible grades, two lots, in two grades, and one lot shows discs in only one grade. Although these samples were judged on poplin discs through which had been filtered only 50 grams of cheese, similar results might have been shown after using the 8-ounce sample and the reversed, milk-sediment, disc.

TABLE 12  
*Macroscopic grades\* of sediment tests (50-gm. samples) from top section of individual cheese*

Lot number	Number of cheese	Distribution of cheese between grades			
		I	II	III	IV
1	12	0	10	2	0
2	5	0	2	3	0
3	10	10	0	0	0
4	10	0	2	7	1
5	9	0	1	4	4

\* Only the grades of Judge A are tabulated.

#### SIZE OF SAMPLE

A sample of satisfactory size should detect the presence of critical material with regularity if it is present and should reveal excessive contamination

in convincing amounts for factory demonstration purposes. In table 13 are summarized the results of paired tests on identical cheese; one test was made using the eight-ounce sample while the other was made with the 50-gram sample. These are the two most commonly used samples in commercial practice.

Table 13 indicates that the 50-gram sample detected the presence of critical material in 37 out of a total 211 samples while the 8-ounce sample detected it in 76. Factories E to H in table 13 indicate irregularity in the ability of both sample sizes to detect critical extraneous material; in some instances one revealed it but the other did not. In the 122 paired tests made on factories A and B, the 8-ounce sample detected critical material in 44 samples in which none was found by the 50-gram sample; the 50-gram sample

TABLE 13

*Relative efficiency of 50-gm. and 8-ounce samples for detecting critical material in cheese*

Factory	Paired tests on identical cheese	Tests showing critical material* under 85× magnification			
		50-gm. sample		8-ounce sample	
		Number	%	Number	%
A	55	20	36	44	80
B	67	10	15	25	37
C	21	2	10	1	5
D	24	1	4	2	8
E	19	2	10	0	0
F	10	0	0	4	40
G	9	0	0	0	0
H	6	2	33	0	0
Totals .....	211	37	17.5	76	36

\* Critical material = material of insect or animal origin.

detected critical material in 9 samples in which the 8-ounce sample failed to catch any. In general, table 13 indicates that the 50-gram sample is half as effective in detecting critical material when 20 per cent or more of the lots tested with this size sample are defective.

It is not necessary to extend this discussion to show detailed comparisons of macroscopic grades of identical cheese tested by using samples of each size. Both are effective tests for control purposes when used with suitable guides made up with actual sediment discs; photographs of such guides are not effective standards. The 8-ounce sample is slightly more uniform because of the greater amount of sediment collected and because of the standardizing effect on particle size of the milk-sediment filter.

The Food and Drug Administration was first to use an 8-ounce sample of cheese; personal conferences with officials of this Administration indicated that this sample was not regarded as either a minimum or a maximum. Although the first reaction of the cheese industry was unfavorable, because of the damage which such large samples would cause when taken from the



cheese, this amount of sample was generally adopted by commercial laboratories with a few exceptions. Two methods of obtaining 8-ounce samples for routine testing were devised which decreased materially the losses caused by plugging the cheese. The first method used a special knife devised by E. Bohacek of the Lakeshire-Marty Company, Plymouth, Wisconsin. This knife cuts an eight-ounce, V-shaped strip of cheese from the flat surface of a cheddar. The cut surface is easily sealed by the paraffining of the cheese. The second method of sampling requires the cooperation of the cheese maker, who is instructed to take a sample of curd just before pressing. This curd, which should be taken from the hoops, is packed solidly into a jar provided with an air-tight closure. If the jar is completely filled and closed the sample can be sent to the warehouse laboratory along with the cheese shipment without danger of spoilage.

Since 50 grams of cheese approximates the yield of cheese from a pint of milk, the amount used in a milk sediment test, it has been suggested (2) that milk sediment standards might be used for judging cheese sediment tests. Factory operations, however, may introduce sediment and Wartinbee (4) reported appreciable amounts of sediment in the whey at dipping; all of which would seem to indicate that the relationship between milk and cheese sediment tests might be misleading.

#### IDENTIFICATION OF EXTRANEOUS MATERIAL ON THE CHEESE SEDIMENT TEST

Identification of extraneous material should classify the material as critical or non-critical. Critical materials are insects or substances of animal origin. Vegetable matter or debris under certain circumstances might also be classed as critical material. Non-critical materials, which, if they are present in appreciable amounts, are indicative of unsatisfactory conditions of milk production or cheese manufacture or both, include: vegetable matter, soil particles, dust, ash, cloth fibers and wood or metal particles.

All types of hair and wool are relatively easy to identify. While hairs of any type are considered critical, hairs from rats and mice are particularly objectionable. Such hairs are distinguished by characteristic markings but it is possible to find hairs from dogs, cats, squirrels and rabbits which so closely resemble rodent hairs that they are practically indistinguishable from them.

Insects or insect parts are critical materials which can be identified more easily if the original insects have been examined carefully. Insects which may be encountered are flies and fly larvae (especially in the soft rinds of cheese like Limburger), cockroaches, centipedes, silverfish, sow bugs, spiders and cheese mites. Insects are chiefly found where poor housekeeping practices prevail.

Cloth fibers which are very apt to appear in cheese sediment tests are the blue cotton fibers from denim overalls. Brightly colored wool fibers prob-

ably come from sweaters worn by those working on the farms or in the factories.

Particles of soil, dust, coal dust, soot, cinders, wood or tobacco ashes may appear on sediment discs. Their origins are obvious and indicate the necessity of better care in the production of the milk or in factory operations. An open window in a factory where smoke from the boiler or dust from a plowed field or dusty road can blow into the plant may easily be responsible for a Number 4 sediment test.

Metal particles, curiously enough, are frequently seen on cheese sediment tests. Probably most of them have their origin in the stirring of milk during cooling but factory operations may also be blamed through scraping of metal equipment and utensils in the making of the cheese. Such contamination might come from knives drawn across the vat, mechanical agitators, curd forkers, curd mills, scoops, rubbing of hoops in the vat and the like.

It is not practical to reproduce here pictures of all kinds of extraneous material which might get into cheese. A fairly complete series of such photographs<sup>2</sup> has been prepared at this laboratory from which prints can be obtained by anyone who is interested in building up a collection to assist in the identification of extraneous matter.

#### SUMMARY

The test for sediment in cheese detects unsatisfactory conditions of milk and cheese production except when the milk supply is filtered efficiently. The results of the test may be graded by macroscopic appearance but should also be examined microscopically for critical material.

The two practical tests use solutions of either sodium citrate or orthophosphoric acid. Commonly eight ounces of cheese is used with the citrate test while 50 grams of cheese is used with the acid solvent. The larger sample must be filtered through a reversed, milk-sediment disc while the smaller sample is most effective when a filter of Irish poplin is used over a milk sediment disc. The smooth surface of poplin can be examined more easily with a microscope since it retains all particles in view; the milk-sediment disc tends to hide all small particles of sediment.

There is not much to choose between the solvents although the acid seems to work faster on young cheese. Neither solvent, under the conditions used in making the tests, destroys enough of the common types of extraneous material to prevent identification although both attack such material more or less. The acid tends to reduce the size and increase the number of sediment particles.

The distribution of extraneous material seems to be fairly uniform within a single cheese but from cheese to cheese from a single lot the distribution is

<sup>2</sup> An index of photographs available for distribution will be sent on request by the Department of Dairy Industry, University of Wisconsin, Madison 6, Wisconsin.

not uniform. Such irregularity makes it desirable to take plugs from several cheese in each lot and essential to test the cheese from a given outlet regularly and as frequently as possible.

The identification of extraneous material by microscopic examination of test pads may provide a useful method of determining sources of contamination. Photographs of common types of extraneous matter which are available for distribution from this laboratory may prove helpful in this respect although they cannot replace microscopic study of the actual material from local sources.

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## THE DETERMINATION OF TANNIC SUBSTANCES IN COMMERCIAL COCOA POWDERS

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### INTRODUCTION

Cocoa and chocolate are extensively used in connection with milk and some of its products, viz., chocolate milk, chocolate ice cream, chocolate puddings, and milk chocolate candies. Recent data (7) indicated a close connection between the content of tannic substances in cocoa powder, as determined by the Ulrich method (8), and its toxicity when cocoa was fed with whole milk powder to white rats. Thus, the tannic substances appear to be of sufficient importance to warrant further investigation of their presence in commercial cocoa powders.

Although the tannic substances of cocoa have not been defined too clearly in the literature, it may be assumed that they may include cacao-tannin, cacao-purple<sup>2</sup> and cacao-brown (1, 5). The amount of these various tannic substances present in commercial cocoa powder depends to a great extent upon treatments, such as fermentation, drying, roasting, and addition of alkali (Dutch process), and to a lesser extent upon the variety of the cocoa bean used.

Various methods have been worked out for the determination of the tannic acid content of plant materials, yet they do not seem appropriate for cocoa products. The writers are not aware of any published methods which will determine accurately the tannic substances totally or separately in cocoa powder. Several methods have been suggested for the determination of cacao-purple. Heiduschka and Bienert (4) do not claim that their method is accurate enough for analytical work. Ulrich's (8) method for the determination of cacao-purple, which is a constituent of the cacao nib but thought to be absent in the shell, was proposed chiefly as a method for detecting

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<sup>2</sup>In this paper cacao-purple is considered as being synonymous with cacao-red. Fincke (2) christened this tannic substance cacao-red, but Knapp (5) considers cacao-purple preferable as this more accurate color description assists in distinguishing it from the oxidation products which are of red-brown color.

cocoa shell in commercial powders. The Ulrich method became the generally accepted test for cacao-purple of cocoa powder. However, Fincke (3) reported this method unreliable for this purpose.

Since the accuracy of Ulrich's method has been questioned, an attempt was made in this study to improve the method. The results obtained are given as a report of progress. Fifteen samples of commercial cocoa powder were analyzed by Ulrich's method before the writers were aware that the accuracy of this method had been seriously questioned. The results are included here, so that they may be compared with analyses of cocoa samples of less recent date, reported by other investigators.

#### EXPERIMENTAL

*Analyses of cocoa powders by Ulrich's method.* Fifteen samples of commercial cocoa powder were analyzed by Ulrich's method. In this method the material to be investigated is boiled with 50 per cent acetic acid, the filtrate is mixed with hydrochloric acid and precipitated by means of ferric chloride, and the precipitate is washed with hot water, filtered off, and weighed. The results of the analyses are summarized in table 1 as average percentages

TABLE 1  
*Average values for ferric chloride precipitates of cocoa powders*

	Original samples	Moisture-fat-free basis
	<i>per cent</i>	<i>per cent</i>
6 Dutch and 9 unprocessed .....	11.01	14.13
6 Dutch samples .....	8.44	10.74
9 Unprocessed samples .....	12.72	16.42
4 Dutch samples when Dutch and unprocessed samples were made from similar base beans .....	8.68	11.27
4 Unprocessed samples when Dutch and unprocessed samples were made from similar base beans .....	12.86	17.06

of ferric chloride precipitate calculated on moisture-fat-free basis and on the original sample.

The average value of 14.13 per cent of ferric chloride precipitate, calculated on a moisture-fat-free basis, approximates the figures reported by other investigators, namely 13.81 per cent by Ulrich (8) and 15.17 per cent by Lythgoe (6). However, the figure for Dutch cocoa is lower than that reported by Lythgoe. Among the Dutch and unprocessed cocoa samples analyzed, four samples of each were made from the same type of cocoa beans and such samples should give a more accurate comparison than samples of unknown history. Obviously, it would be desirable to have samples of Dutch and unprocessed cocoa which were made from the same lot of beans, but sufficient samples of this type were unavailable. The average percentage of ferric chloride precipitate was approximately one-third less for the

Dutch cocoa than for the unprocessed cocoa. Moisture and fat in the cocoa samples ranged from 1.09 to 5.32 per cent and 10.10 to 23.71 per cent respectively.

*Modification of Ulrich's method.* Since Ulrich does not indicate the basis of his method or the nature of the material brought to precipitation, the precipitated material is usually referred to simply as "ferric chloride precipitate." Some investigators, however, have reported the amount of ferric chloride precipitate as if it consisted entirely of tannic substances.

Numerous ferric chloride precipitates were analyzed in this study in an attempt to secure some information on the nature of the material precipitated; also, the effect of altering the procedure for washing the precipitate was studied. The results are given in table 2. The ferric chloride precipitates, washed according to Ulrich's method and ashed, had an average ash content of 13.45 per cent, which varied from 11.48 to 18.24 per cent. Analyses of the ash showed it to consist mainly of iron and phosphorus, present either as separate oxides or in combination as ferric phosphate. The relatively high ash content of the ferric chloride precipitate indicated that the weight of the precipitate cannot be taken as a true measure of cacao-purple or other tannic substances, because the value is too high, at least to the extent of the weight of ash in the precipitate.

In order to determine whether the ash content could be reduced, thereby making the weight of the ferric chloride precipitate more nearly a true measure of the amount of tannic substances present in cocoa powder, it was decided to alter the portion of the Ulrich procedure which pertained to the washing of the precipitate. Qualitative tests made on a number of precipitates, which had been washed with hot water according to the Ulrich method, showed the presence of small amounts of chloride, indicating that some of the iron appearing in the ash probably was due to incomplete removal of ferric chloride by washing. Two modifications in washing the precipitate were used. Some precipitates were washed first in cold acetic acid (1+1) and then in hot water. Other precipitates were washed with a cold mixture of acetic and hydrochloric acids of the same concentration as used in extracting the tannic substances, the final washing likewise being with hot water. In general, washing the precipitate with either acid solution resulted in a lower percentage of ash in the precipitate and a lower percentage of iron and a higher percentage of phosphorus in the ash. However, the mixture of acetic acid with hydrochloric acid was more effective than the acetic acid alone. Whatman No. 42 filter paper and a filter crucible were also compared as to their desirability for use in collecting and washing the precipitate. The washing was slightly more effective when the filter crucible was used, but the difference appeared too small to be of any great significance. It was also noted that the proportion of iron to phosphorus approached the theoretical proportions existing in ferric phosphate, when the precipitates were washed with acid.

*Analyses of ferric chloride precipitate*

Sample No.	Brand No. and kind of cocoa	Treatment of precipitate	Per cent FeCl <sub>3</sub> ppt.		Per cent ash in ppt.	Per cent Fe in ash	Per cent P in ash	Per cent Fe and P in ash calculated to per cent FePO <sub>4</sub>	Excess Fe in ash calculated to per cent Fe <sub>2</sub> O <sub>3</sub>	Excess P in ash calculated to per cent P <sub>2</sub> O <sub>5</sub>
			On dry fat-free basis	On original sample						
1	II Dutch	According to Ulrich's method*	15.91	11.95	11.95	40.6	.....	.....	.....	.....
2	II Unprocessed	" " " "	17.77	13.08	11.48	39.2	.....	.....	.....	.....
3	III Dutch	" " " "	17.17	13.84	11.95	42.3	15.6	75.9	20.3	.....
4	III Unprocessed	" " " "	16.29	11.57	12.76	41.4	17.1	83.1	15.2	.....
5	IV Dutch	" " " "	7.42	5.43	18.24	44.8	14.6	71.0	26.4	.....
6	IV Unprocessed	" " " "	16.50	13.35	12.78	45.2	15.0	72.9	26.0	.....
7	XII Dutch Liquor	" " " "	10.28	4.55	17.48	42.2	13.4	65.2	25.9	.....
8	Unprocessed Liquor	" " " "	16.02	6.95	14.51	40.8	14.6	71.0	20.7	.....
9	Unprocessed	" " " "	16.53	13.54	11.55	45.6	15.1	73.4	26.3	.....
9a	"	Used Whatman No. 42 filter paper washed ppt. with cold 50-50 acetic acid. Finished washing with hot water	15.71	13.06	9.80	39.3	18.6	90.4	8.3	.....
9b	"	Used filter crucible. Washed ppt. same manner as for 9a	16.55	13.80	9.50	38.3	18.1	88.0	8.1	.....
10	X	According to Ulrich's method*	13.78	10.91	11.80	45.4	15.3	74.4	25.6	.....
10a	"	Used Whatman No. 42 filter paper washed ppt. with cold acetic acid + HCl acid. Concentration of acid mixture same as precipitating solution. Finished washing with hot water	13.53	10.50	7.51	33.3	22.8	90.0	.....	9.8
10b	"	Used filter crucible. Wash ppt. in manner as for 10a	13.57	10.67	7.13	31.6	23.1	85.4	.....	12.6



## SUMMARY AND CONCLUSIONS

1. Fifteen samples of commercial cocoa powders were analyzed, presumably for cacao-purple, by Ulrich's ferric chloride precipitate method. The ferric chloride precipitates ranged from 3.48 to 15.59 per cent, with an average of 11 per cent. The average percentage of ferric chloride precipitate obtained from Dutch cocoas was approximately one-third of the amount obtained from the unprocessed cocoas.

2. The amount of ferric chloride precipitate does not measure accurately the amount of cacao-purple or other tannic substances in cocoa, partly because the ash content of the precipitate was found to be relatively high, averaging 13.45 per cent.

3. The ash consisted mainly of iron and phosphorus, present either as separate oxides or in combination as ferric phosphate.

4. A modification of the method of washing the ferric chloride precipitate has been suggested, which reduced its ash content.

5. Results obtained from this study indicate that the ferric chloride precipitate would measure the tannic substances content of cocoa more accurately if the modification of the washing procedure were followed and correction made for the ash contained.

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# THE RECOGNITION OF THE INFLUENCE OF AGE ON BUTTERFAT PERCENTAGE WHEN CALCULATING MATURE EQUIVALENTS\*

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## INTRODUCTION

The use of conversion factors in calculating dairy cattle production records to standard equivalents has become an accepted procedure. General recognition has been given to the fact that as dairy cows increase in age their milk and butterfat production increases until senescence brings about a gradual decline from year to year. It has also been shown (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17) that fat percentage travels a related but inverse line. Fohrman (4) and Heizer (13) have also shown that the milk and butterfat production for dairy cows increases at a dissimilar rate which justifies different age conversion factors for milk and butterfat. The results of these studies, however, have been given no consideration by the various agencies that report production records on a standardized equivalent basis. The present procedure is to multiply the actual milk production by a factor, and then multiply the actual butterfat production by the same factor. It is the purpose of this paper to offer additional proof of the effect of age on the butterfat percentage in the milk of dairy cows, and to point out the error resulting from the use of the present method of calculating mature equivalents for milk and butterfat production.

## PRESENTATION OF DATA

In 1941 the Ayrshire Breeders' Association placed all of its production records on punch cards and approved the use of these cards for analytical purposes by the Department of Dairy Husbandry of the West Virginia Agricultural Experiment Station. The first 34,176 of these records are involved in this study. These records were for complete lactations not exceeding 305 days in length. They were sorted on the basis of the age of the cow at the beginning of the lactation.

The effect of age on the butterfat percentage in the milk of Ayrshire cows is shown in table 1. This table contains the number of animals in each age group, the actual butterfat percentages for the different ages, and conversion factors calculated from these various percentages.

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TABLE 1

*Butterfat tests of Ayrshire cows at different ages and corresponding age conversion factors*

Age of cow beginning of record (years)	Number of records for each age group	Average butterfat percentage	Butterfat percentage differentials	Age conversion factors for per cent butterfat
2	8,238	4.09	-0.10	0.9755
3	6,794	4.07	-0.08	0.9803
4	5,305	4.05	-0.06	0.9852
5	4,037	4.02	-0.03	0.9925
6	3,123	3.99	.....	1.0000
7	2,302	3.96	+0.03	1.0007
8	1,637	3.94	+0.05	1.0130
9	1,185	3.91	+0.08	1.0200
10	762	3.90	+0.09	1.0230
11 & over	1,114	3.87	+0.12	1.0310

The accompanying figure shows the regression of butterfat percentage on age of cow at the beginning of the record.

## DISCUSSION

The variation in the per cent butterfat in the milk of Ayrshire cows at different ages, as shown in table 1, suggests the necessity of a change from the present method of calculating mature equivalent production records. Practically all conversion factors in use were worked out for total butterfat, rather than for milk. It has become the general practice, however, to apply these butterfat conversion factors to both milk and butterfat. By this method an error is introduced which, although considered insignificant by some investigators (6, 14, 15, 17), is, according to the data involved in this study, statistically significant and is of importance economically both in a breeding program and in a dairy enterprise.

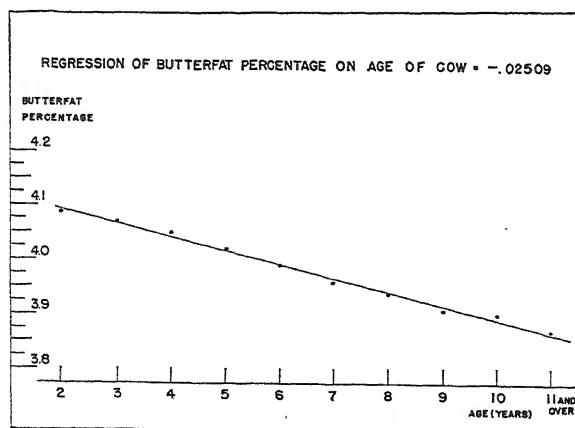


FIG. 1

Conversion factors are a necessary evil to which we must resort in making mature equivalent production comparisons of dams and daughters in evaluating sires. It is a recognized fact that when applied to individual records conversion factors may err greatly as an indicator of mature performance. Deliberate applications of conversion factors to individual production records in such a way as to further increase the amount of possible error, however, should, without question, be avoided.

Since it is known that the fat percentage of cows' milk decreases with the advancing age of the cow, the application of conversion factors, developed for total butterfat, to butterfat percentage will introduce an error that is always made in the same direction. Furthermore the application of those conversion factors, which were developed for total butterfat, to total milk production also introduces an error that tends to be always in the same direction. The assumption that the first lactation butterfat percentage also applies to mature cows is to give bulls credit, in mature equivalent calculations, for transmitting a higher fat percentage than is really the case. Furthermore, the application of the same factor to the first lactation milk production, in raising it to a mature equivalent, does not credit the sire with having transmitted as much total milk as he actually did transmit. The final result of such applications of the butterfat conversion factor for all three conversions is that a sire is credited with the correct amount of total butterfat but with a butterfat percentage impossible of attainment and with a total mature equivalent milk flow of less than he transmitted. These facts are apparent from a study of the following example, where a cow makes a record at 2 yrs. 6 mos. of age of 10,000 pounds of milk testing four per cent and 400 pounds of butterfat. Applying the standard U.S.D.A. Bureau of Dairy Industry butterfat factor of 1.211 to this record a mature equivalent of 12,110 pounds of milk testing four per cent and 484 pounds of butterfat is obtained. Applying the fat percentage differential of one tenth of one per cent as indicated in table 1, however, the mature equivalent butterfat percentage would be 3.9 per cent instead of four per cent. By dividing 3.9 per cent into the total mature equivalent butterfat of 484 pounds a mature equivalent milk production of 12,410 is indicated. We find then that this cow that would have been credited with 12,110 pounds of milk, testing four per cent, and 484 pounds of butterfat at maturity should actually have a mature equivalent of *12,410 pounds milk, testing 3.9 per cent*, and 484 pounds of butterfat. This difference of 300 pounds of milk, since it is always going in the same direction, cannot be ignored although it may not be statistically significant. For those agencies that include equal parent indices in proved sire reports, the difference will be slightly more important.

An even greater hazard in the failure to recognize the decline in butterfat percentage with age lies in the fact that the owners of those breeds of

cattle in which the maintenance of a minimum fat percentage is important, are lulled into a false sense of security in selecting proved herd sires. In selecting a four per cent bull to use on his herd that actually averages four per cent the prospective purchaser does not know that the bull whose dam-daughter comparison indicates a four per cent level (by present methods) is actually a 3.9 per cent bull. In fact this may be one of the reasons why, despite the efforts to select for higher test in the lower testing breeds, so little progress has been made in increasing the average butterfat percentage for these breeds even in herds where a testing program is carried on.

Any agency reporting dam-daughter comparisons on a mature equivalent basis for the evaluation of sires has assumed a serious obligation. Every effort should be made to reduce to a minimum the possibilities of errors due to method. The use of factors for both total milk production and total butterfat production, or the recognition of fat percentage differentials, properly applied if a single set of conversion factors are used, should help greatly to keep variations from the actual production trend with age at a minimum.

#### SUMMARY

A study of 34,176 lactation records not exceeding 305 days in length shows a definite decrease in the butterfat percentage of the milk of Ayrshire cows from the first lactation to old age that is statistically significant. Because of this decline in butterfat percentage with age it is suggested that it would be more nearly accurate to use age conversion factors for both milk and butterfat production, or to use a fat percentage differential as a correction factor when using only one set of age conversion factors for both milk production and butterfat production.

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# TEMPERATURE-TIME RELATIONSHIPS FOR HIGH-TEMPERATURE-SHORT-TIME STERILIZATION OF EVAPORATED MILK

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High-temperature-short-time sterilization is used commercially in this country only for canned fruit and vegetable juices. The application of this method of sterilization to the processing of evaporated milk presents certain problems, which arise chiefly from the chemical and physical instability of the product in storage. In studying these effects, which have been reported in another paper (2), the writers also obtained data on the temperature-time relationships for the death points of spores. These data are presented in this paper.

## METHODS AND EQUIPMENT

The general procedure consisted of the inoculation of concentrated whole milk with washed spores of the desired level of heat resistance and the heating of the concentrate at different high temperatures for periods of seconds followed by rapid cooling. Examination of the aseptically collected milk and of plated control and heated samples after an appropriate period of incubation revealed the course of destruction of the spores.

The test organism (9499) was an unidentified sporing species isolated from spoiled, commercially canned, evaporated milk. It is a facultative thermophilic aerobe; growth optimum 37°–40° C. (98.6°–104° F.), maximum 50°–55° C. (122°–133° F.). In evaporated milk (26% solids) at 115° C. (239° F.) the thermal death time of the spores (100,000 per ml.) was approximately 14 minutes.

The spores were produced on nutrient agar slopes. After 3 weeks of incubation at 37° C., the growth—practically 100 per cent spores—was collected, washed 3 times by centrifugation with sterile distilled water, and filtered through cotton. The clumps were largely dispersed by agitating their aqueous suspensions with small glass beads. Suspensions of the spores in distilled water were stored at 6° C. (42.8° F.).

Samples of the inoculated milk taken before and after heating were plated with glucose extract agar and the colonies counted after 48 hours at 37° C. Flasks of aseptically collected milk, tightly stoppered, were incubated at 37° C. and examined at weekly intervals for 3 months. Samples of the uninoculated milk also were plated, both before and after heating at 85° C. for 10 minutes. The concentration of viable spores in the milk before the heat treatment was approximately 100,000 per ml., of which less

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than 30 had been present prior to inoculation. Since the test spores were found to be susceptible to heat-activation,<sup>1</sup> the total viable count was considered to be that obtained by the plating of the inoculated milk after heating at 95° C. for 10 minutes.

The sterilizer was a Mallory small-tube heat-exchanger (3) consisting of a heating section and a holding and cooling section integrally combined and connected to a high-pressure reciprocating pump which forced the milk through the small tubes at high velocity. The temperature was raised by introducing high pressure steam through a reducing valve into the heating chamber and the holding time was varied by changing the length and diameter of the tubing through which the hot milk was forced enroute to the cooling coil.

The cooled milk was discharged aseptically into sterile bottles by means of a special collecting device; this consisted of a glass manifold which could be connected without internal contamination to the by-pass outlet of the heat-exchanger. Half-pint milk bottles and two large flasks were attached to this manifold by rubber tubing. Each container was closed with a rubber stopper fitted with two short lengths of glass tubing. One of these served as an air vent, the other was connected to the manifold by means of rubber tubing. Open ends of the sample-collecting assembly were plugged with cotton and covered with paper held in place with rubber bands.

Individual parts of the sampling assembly were cleaned, assembled, sterilized in an autoclave, carried as a unit to the heat-exchanger, and aseptically attached to the by-pass outlet. The heating and cooling coils, and each holding tube, were sterilized by hot water heated in the heating coil to 120° C. (248° F.) or higher, directed into the connecting and holding tubing and thence into the cooling coil. The latter was not surrounded by water while it was being sterilized. Means were provided for creating a back pressure in the cooling coil sufficient to insure sterility in 20 minutes.

By manipulation of pinchcocks the processed milk was directed from the main line of the assembly through the glass manifold into the sterile sample containers. When these were filled to the desired level the manifold was detached from the sampling outlet, plugged with sterile cotton, and the entire assembly carried to the plating room. Samples to be used for bacteriological examination were incubated directly or subcultured as previously described. The remainder of the samples were used in the study of the physical properties of the milk (2). With these samples the original perforated stoppers were aseptically replaced with sterile solid rubber stoppers, the bottles labeled, covered with small sterile paper bags and stored with tinned samples at 30° C. The nature and general arrangement of the processing and sampling equipment are shown in figure 1.

<sup>1</sup> A discussion of this subject will be prepared for publication elsewhere.

## RESULTS

The essential bacteriological data are summarized in the accompanying graphs (figures 2 and 3). Figure 2 shows the minimum time in seconds required for the killing of all the test spores at four different temperatures.

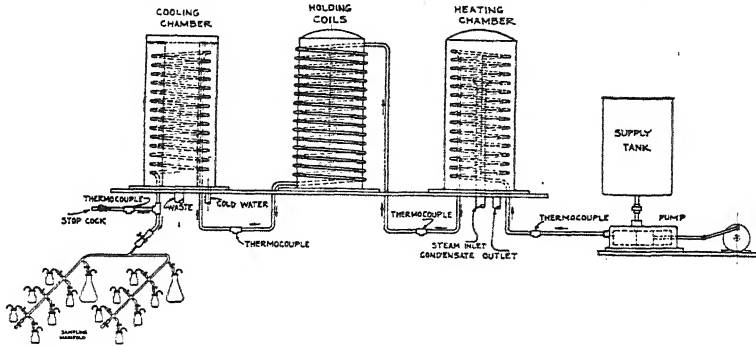


FIG. 1. Schematic drawing of the processing and sampling equipment.

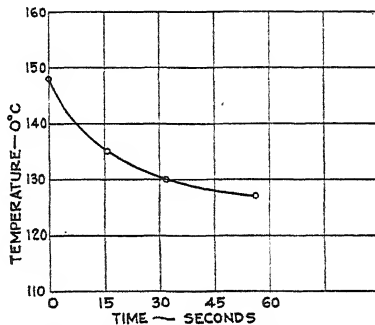


FIG. 2. The relationship between temperature and time for sterilization.

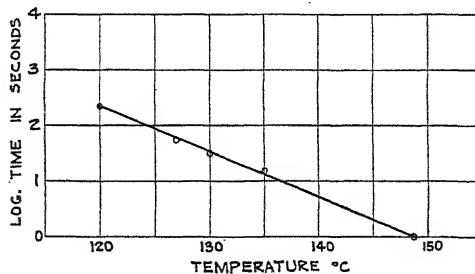


FIG. 3. The relationship between temperature and the logarithms of the sterilizing time.

Since approximately 3 seconds was required for heating and also for cooling, zero holding-time heating actually involved about 6 seconds of rapidly changing temperatures between 30° C. (86° F.) and 148° C. (298.4° F.).

This period of changing temperatures is estimated to cause less change in actual data than can be accurately plotted in the scale chosen. In view of the heat-resistance capacity of the test spores, these data afford a valid index of the sterilizing efficiency of the heat-exchanger for evaporated milk. Figure 3 shows the linear relationship between the temperature and the logarithms of the sterilizing time.

The two factors which precisely define a thermal death curve—viz., the thermal death time at a specified temperature and the thermal death curve slope (interval in temperature per log cycle)—have been utilized by Ball (1) to develop mathematical equations for estimating the lethal effect of sterilization processes. Since information is lacking concerning the constancy of the slope of the thermal death curve at temperatures above 130° C. (260° F.), the applicability of these equations to such temperatures is held to be uncertain. The small-tube, continuous-flow heat-exchanger which provides extremely rapid and uniform heating, should be useful in obtaining the necessary experimental data for the clarification of this subject.

The reported results were obtained in evaporated whole milk of 26 per cent solids content. Increasing the solids to 32 per cent slightly increased the required sterilizing time. Variations in forewarming treatment produced no measurable change in the sterilizing time. Throughout the range in treatments represented in figure 2, the color, flavor, and odor of the resulting product was superior to that of the conventionally sterilized product. The development of certain non-microbial physical and chemical changes incident to long storage presents the most serious problem in the successful application of high-temperature-short-time sterilization of evaporated milk (2).

#### SUMMARY

The temperature-time relationships for the destruction of spores in concentrated whole milk by high-temperature-short-time heating are given and the method described.

The required killing time is an exponential function of the temperature.

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# EFFECTS OF TEMPERATURE AND TIME OF STERILIZATION UPON PROPERTIES OF EVAPORATED MILK

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The effects of high-temperature-short-time heating of milk and of its concentrate on the heat stability of the concentrate have been described in recent publications (7, 8). The work reported here deals with the high-temperature-short-time<sup>1</sup> sterilization of concentrated milk in its relationship to some of the properties of the evaporated product and compares these properties with those of evaporated milk made in the conventional manner.

## METHODS AND EQUIPMENT

The source of milk, the standardization of the milk to a fat to solids-not-fat ratio of 1:2.29, forewarming, high-temperature heating, condensing, homogenizing, pilot sterilizing and cooling were as described by Webb, Bell, Deysher and Holm (8).

New facilities included means for aseptically packaging the high-short sterilized milk. They are described by Curran, Bell and Evans (2).

Viscosity measurements were made with a MacMichael viscosimeter and the values converted to centipoises. All measurements were made at 30° C., the first within 3 days after the milk was sterilized.

In preparing controls, the sterilizing temperature was 115° C. (239° F.) and the holding time 18 minutes.

The sterilizing efficiency of the high-short process for evaporated milks was determined by pretreatment inoculation of the milk with washed spores of suitable heat resistance followed by incubation or subculture of the heated samples (2).

Some of the high-short sterilized evaporated and the unsterilized control concentrates were canned and heated in a pilot sterilizer at 115° C. for various periods, cooled in a few minutes and stored at 30° C. with the other samples.

The methods of measuring color were those used by Webb and Holm (9).

## RESULTS

The influence of the forewarming treatment, solids content and sterilizing conditions on the heat stability, viscosity and color of evaporated milks made from well-mixed portions of the same raw milk are shown in table 1.

The forewarming treatment had a striking effect on the heat stability of the samples. A forewarming treatment of only 65° C. (149° F.) for 10

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<sup>1</sup> Hereafter designated high-short sterilization.

TABLE 1

*The effects of forewarming treatment, solids content, and sterilizing conditions on the heat stability, viscosity, and color of evaporated milks*

Forewarmed		Total solids	Sterilized		Heat stability	Viscosity at 30° C.	Color
At	For		At	For			
° C.	min.	per cent	° C.	min.	min.	centipoises	
95	10	26.0	.....	.....	27	9.0	1
95	10	26.0	115	18.0	.....	14.5	4
95	10	32.5	.....	.....	15	.....	1
95	10	32.5	115	18.0	.....	260.0	6+
65	10	26.0	.....	.....	3	7.0	1
65	10	26.0	135	0.5	53	8.0	1
65	10	32.5	.....	.....	0	10.5	1
65	10	32.5	135	0.5	16	15.0	1+
120	4	26.0	.....	.....	84	7.0	1
120	4	26.0	135	0.5	95	8.5	1+
120	4	32.5	.....	.....	35	14.5	1
120	4	32.5	135	0.5	43	17.5	2-

minutes, although sufficient to protect the milk against the development of rancidity, did little to stabilize its concentrate to heat.

However, after this concentrate had been sterilized by the high-short method at 135° C. (275° F.) for 30 seconds its heat stability was 53 minutes instead of 3 for the 26.0 per cent evaporated and 16 minutes instead of 0 for the 32.5 per cent solids content product.

Under appropriate conditions, 120° C. for 4 minutes is an unusually effective milk forewarming treatment for increasing the heat stability of concentrated milk (8). Concentrates of this milk that contained 26.0 and 32.5 per cent solids had a heat stability of 84 and 35 minutes, respectively. After sterilization by the high-short method these values were increased only to 95 and 43 minutes, respectively.

The evaporated milk which had a viscosity of 260 centipoises at 30° C. curdled during sterilization because the time necessary to initiate coagula-

TABLE 2

*The effect of heating high-short (135° C.—0.5 minute) sterilized evaporated milks in cans at 115° C. for 2, 6, 12 and 18 minutes on their viscosity and color*

Milk solids	Time at 115° C.	Whole milk forewarmed at 65° C. for 10 min.		Time at 115° C.	Whole milk forewarmed at 120° C. for 4 min.	
		Viscosity	Color		Viscosity	Color
per cent	min.	centipoises		min.	centipoises	
26.0	2	8.0	1+	2	8.0	2
26.0	6	9.0	2	6	8.5	3-
26.0	12	9.5	2+	12	9.0	3+
26.0	18	10.0	4+	18	9.5	5
32.5	2	19.0	2-	2	16.0	2
32.5	6	25.5	2	6	17.5	3-
32.5	12	59.0	4	12	21.5	5
32.5	18	278.0	6+	18	27.0	7+



tion in this 32.5 per cent concentrate at 115° C. was only 15 minutes. The color value of this sample and that of the 26.0 per cent evaporated milk prepared in the customary manner was high. The color of the unsterilized milks was only slightly increased by high-short sterilization.

The effect of sterilizing conditions which are a combination of the high-short method of these experiments and of those usually practiced on the viscosity and color of the milks is shown in table 2. The high solids evaporated milk made from high temperature forewarmed whole milk, being relatively stable to heat, retained much of its fluidity even after 18 minutes in a can at 115° C. Of further interest in this table are the data which show that when the high-short sterilized milks were heated in the cans at 115° C. for 6 minutes their color was not greatly increased. Most of the

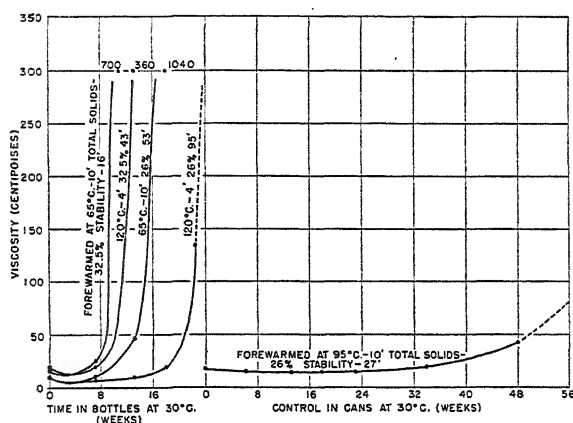


FIG. 1. Effect of widely different forewarming conditions and solids content on the viscosity of high-short evaporated milks during storage. Forewarming treatment of whole milk, solids content, and heat stability of evaporated milk are shown on each curve. Bottled concentrate was sterilized by heating at 135° C. for 30 seconds. Control concentrate of 26% total solids content was sterilized in cans by heating at 115° C. for 18 minutes.

darker color of the samples heated 18 minutes at 115° C. was imparted during the last one-third of this period (see also Webb and Holm, 9).

In figure 1 are shown the viscosity changes during storage at 30° C. in the high-short sterilized and bottled samples referred to in table 1. The contents of these bottles began to lose their fluidity in from 6 to 16 weeks, depending on the method by which they were prepared. Thereafter these milks thickened rapidly and, in the course of a few more weeks, formed a gel.

The samples referred to in figure 2 were high-short sterilized and then heated in the small cans at 115° C. for the minutes marked on each curve. These curves, together with those in figure 3 reveal not only the stabilizing effect of the additional heat treatments in the cans but the influence of initial heat stability on stability during storage.

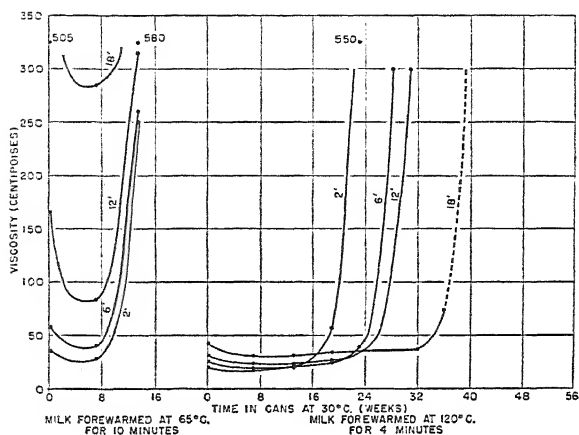


FIG. 2. Effect of time of heating at  $115^{\circ}\text{C}$ . in cans upon the viscosity during storage of high-short evaporated milks of 32.5% solids content referred to in figure 1. Minutes at  $115^{\circ}\text{C}$ . are shown on curves.

Figure 3 is similar to figure 2 except that it refers to evaporated milks of the composition of the article of commerce. The viscosity or body of the samples prepared from high-short sterilized evaporated milk of unusually high heat stability (95 minutes) increased at a relatively slow rate. The viscosity values of these samples remained low twice as long as did those of samples of lower heat stability (53 minutes) shown in the same figure.

#### DISCUSSION

The work reported here demonstrates the relative ease with which fresh evaporated milk of lighter color can be prepared. This lighter color was

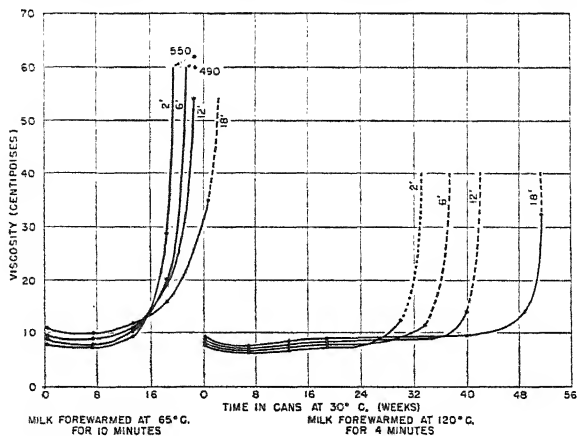


FIG. 3. Effect of time of heating at  $115^{\circ}\text{C}$ . in cans upon the viscosity during storage of high-short evaporated milks of 26% solids content referred to in figure 1. Minutes at  $115^{\circ}\text{C}$ . are shown on curves.

accompanied by a decrease in the intensity of the cooked flavor. Furthermore, these improvements in color and flavor were permanent, that is, they distinguished the aged as well as the fresh high-short sterilized samples from those sterilized by heating at 115° C. for 18 minutes.

None of the samples examined during the course of the experiments discussed in this paper had an oxidized or rancid flavor.

An important difference in the evaporated milks made by the two methods is in the properties of their cream layers. In the high-short sterilized samples, and particularly those of low heat stability, as storage changes progressed, the cream layer became jellylike, well defined, and deep. It was of short texture in that it broke cleanly and smoothly as well-firmed cheddar cheese curd does at cutting time. As the heating period in the cans was lengthened the cream layer which developed during storage was less well defined. It was softer and creamier and the fat in it could be redispersed with relative ease.

Grindrod (3) recognized the beneficial effect of additional heating of quickly sterilized evaporated milk on fat separation during storage in his U. S. Patent No. 1,714,597 (1929). On page 9, in lines 83 to 90, he said, "After such sealing, the filled cans are placed in a sterilizer of suitable form, such as now used for sterilized milk, and are given a heat treatment for say ten minutes at 230° F., which serves to destroy any accidental bacterial contamination and to bring about the colloidal adsorption which prevents separation or churning of the fat."

The high-short treated milks developed a gel which, when broken before it became firm, separated into curds and whey. As the body of the samples made in the conventional manner slowly became a gel the material held its water better than did that of the high-short samples and, when poured or stirred, was more homogeneous.

A factor which is of importance to the manufacturer of evaporated milk is the ability to control the heat stability and to a large degree thereby the body of his product. To do this the manufacturer strives to attain a concentrate which has a heat stability of a few minutes longer than to about twice that of the sterilizing period. Then, by varying the sterilizing conditions, he can approach the heat stability limit and attain a more viscous milk. The greater viscosity retards fat separation during storage and gives the appearance of richness to his product.

During high-short sterilization there is little opportunity to control body, and, through a heavier body, the extent of fat separation.

The difference in storage stability between evaporated milks made by the two methods of sterilization presumably was due primarily to the greater effect which the longer time of heating at the lower temperature had on the casein as it existed in the concentrates. The longer time of heating at the lower temperature apparently made the caseinate less hydrophylic and to this extent caused the less fluid sols to be more stable.

It is possible that the calcium and magnesium salts, because of the unique effect of heat on their solubilities, had a minor influence. Mojonner and Troy (4) state that tricalcium citrate is more easily soluble in cold than in hot water and that after evaporated milk has aged for a considerable time there appears upon the bottom of the cans white, gritty, sand-like particles which are lime salts of citric acid or tricalcium citrate,  $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2 + 4 \text{H}_2\text{O}$ . Sato (6) ascertained that the small white particles which he found on the bottom of a two-year-old specimen of evaporated milk were composed of tricalcium phosphate,  $\text{Ca}_3(\text{PO}_4)_2$ , magnesium phosphate,  $\text{Mg}_3(\text{PO}_4)_2$ , and tricalcium citrate,  $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ . According to Pollacci (5) one liter of rain water dissolves 0.0216 gram of tricalcium phosphate at  $12.5^\circ$  and 0.0120 gram at  $100^\circ$ . Chatterjee and Dhar (1) found that at  $30^\circ$ , 2.51 grams of  $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2 + 4 \text{H}_2\text{O}$  will dissolve in one liter of water and only 2.10 grams at  $95^\circ$ .

When solutions of these salts are heated, considerable time is required for attainment of the new solubility equilibrium. When concentrated milk is sterilized by the high-short method the conditions are less favorable for attainment of the new salt equilibrium than during the low-long procedure and presumably less calcium and magnesium are rendered insoluble. Hence, the tendency for the protein particles to swell and form a gel might be greater.

Experiments have been completed which differed in the high-short sterilizing treatment and other details but not in the pattern of those reported above. Holding periods of a fraction of a second, 15, and 60 seconds at temperatures of 150, 140, and  $125^\circ \text{C}$ ., respectively, were employed. Although the results of these experiments likewise varied as to detail, they do not change the general conclusions.

#### SUMMARY

Highly heat stable high-temperature-short-time sterilized evaporated milks of 26 per cent solids content resembled the unsterilized product in color, were only mildly cooked in flavor, and were of light body (low viscosity). When stored at  $30^\circ \text{C}$ . they were of fairly satisfactory quality for only approximately 4 months.

Brief heating in cans at  $115^\circ \text{C}$ ., increased the storage life of these high-short sterilized milks. After 18 minutes at this temperature they were as stable in storage as evaporated milk made by forewarming the whole milk at  $95^\circ \text{C}$ . for 10 minutes and sterilizing its 26 per cent solids concentrate at  $115^\circ \text{C}$ . for 18 minutes.

In the manufacture of evaporated milk, sterilization at  $115^\circ \text{C}$ . for 18 minutes not only destroys the contained microorganisms and affords an opportunity to control the body but it also decreases the firmness of the cream layer which forms during storage and increases the ease with which the fat in this cream layer may be redistributed in the product.

Long continuing fluidity and high heat stability in evaporated milk are not correlated. Long continuing fluidity is rather due to changes caused by heat which are associated with a darker color and a more cooked flavor.

The solution of problems associated with instability of the product on long storage must precede the commercial application of high-short sterilization in the manufacture of evaporated milk.

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## A NEW METHOD FOR INDEXING DAIRY BULLS

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Dairy cattle breeders use many criteria for evaluating breeding worth in their animals. Among the best criteria and in descending order of merit, stand (A) Progeny, (B) Performance, (C) Pedigree. It is generally accepted that the best measure of an animal's breeding or transmitting value is the sort of progeny the animal begets. This is especially true in the case of dairy bulls, since they themselves yield no product. With the cow we can get performance (a record or records) and relatively few progeny. With the bull we cannot get performance in this sense but may get many progeny.

For a considerable time dairy bulls were judged on the basis of their daughters' production alone. During the past 25 years various methods of indexing dairy bulls by comparing their daughters' production with that of the bull's mates (or daughters' dams) have been proposed (1-21). Hansson, Woodward, Yapp, Gifford, Pearl, Gowen, Turner, Goodale, La-Master, Wright, Heizer and Norton have contributed ideas on the indexing of dairy bulls. Lush (12), in 1933, discussed the general genetic problems involved in indexing including the idea of invoking regression proposed by H. W. Norton, Jr., in some unpublished data.

Heizer (9), in 1933, on the basis of his analysis of the extended records in a large Ayrshire herd, suggested the use of regression toward the herd or breed average in indexing bulls and in predicting the production of daughters from dams with records and by indexed bulls. Yapp (20), in 1938, discussed the relation of regression to selection.

Many investigators have reported daughter-dam correlations in dairy cattle both for amount of milk and butterfat test. These correlations have ranged from about 0.2 to 0.5 depending on whether they referred to individual or group correlations, the source of the data and the intensity of selection practiced on the daughters or dams, and perhaps on still other circumstances.

The writer has restudied the problem of daughter-dam correlations especially as it relates to the indexing of dairy bulls. Five of the major dairy breeds in America were included in the study which involved the following numbers of daughter-dam comparisons:

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Ayrshire .....	19,885
Brown Swiss .....	808
Guernsey .....	1,754
Holstein .....	23,706
Jersey .....	1,656

First a study was made of individual records. The Ayrshire Breeders' Association made available about 3000 daughter-dam comparisons taken at random from the breed files at Brandon, Vermont; and the Holstein-Friesian Association of America made available about 3500 daughter-dam comparisons chosen at random from the breed files at Brattleboro, Vermont.

Correlation surfaces of this material were prepared and yielded the data found in table 1.

TABLE 1

*Individual daughter-dam comparisons for amount of milk and butterfat test, Ayrshire and Holstein*

	Mean	Standard deviation	Correlation	Regression daughters on dams
Ayrshire Milk, Dams .....	8,938	1,832	$0.289 \pm 0.017^*$	0.291
Daughters .....	8,784	1,846		
Holstein Milk, Dams .....	12,050	3,036	$0.322 \pm 0.015$	0.333
Daughters .....	12,182	3,147		
Ayrshire Butterfat Test, Dams .....	4.07	0.322	$0.482 \pm 0.014$	0.474
Daughters ..	4.08	0.317		
Holstein Butterfat Test, Dams .....	3.46	0.277	$0.433 \pm 0.013$	0.436
Daughters...	3.51	0.279		

\* Figures throughout paper are standard errors.

From table 1 it is evident that individual daughter-dam correlations for amount of milk is about 0.3 and for butterfat test about 0.45. These correlations may include much or little from common environment in addition to what they include from heredity in the true sense; *i.e.*, from the fact that daughter and dam have half their genes identical and may resemble each other in the remaining half as much or little as unrelated members of the same breed would. In most cases daughter and dam are tested in the same herd. If the environment of that herd is better than average, both records are likely to be raised; if the environment in that herd is less favorable than average, both records are likely to be low. Thus the amount of the environmental contribution to the daughter-dam correlation depends both on how widely the average environment differs from herd to herd in the material being studied, and on how much influence those environmental differences have on the characteristic (milk might be more labile than test, for example) being studied.

#### GROUP COMPARISONS

Next a study was made of groups of dams and their daughters by individual bulls—such data as are commonly used in indexing dairy bulls. Data



TABLE 2  
Group daughter-dam comparisons for amount of milk

	Number bulls	Daughter-dam comparisons	Mean milk	Standard deviation	Correlation	Regression of daughters on dams
Ayrshire .....	73	540	Dams ..... 8,455 Daus. .... 8,142	1,172 1,207	0.479 ± 0.090	0.493
Brown Swiss	109	808	Dams ..... 9,370 Daus. .... 9,606	1,350 1,467		
Guernsey .....	225	1,754	Dams ..... 7,676 Daus. .... 7,730	1,010 1,139	0.617 ± 0.041	0.695
Holstein .....	561	4,635	Dams ..... 11,062 Daus. .... 11,288	1,547 1,660		
Jersey .....	234	1,656	Dams ..... 7,234 Daus. .... 7,073	1,051 1,083	0.619 ± 0.040	0.637 Av. 0.601

printed in U.S.D.A. Miscellaneous Publications No. 522, May, 1943, provided part of the material for analysis and yielded the data listed in tables 2 and 3.

It is obvious from tables 2 and 3 that higher correlations and regressions result when animals are taken as groups, as is done in indexing dairy bulls. As individuals, the correlations for amount of milk and butterfat test average about 0.3 to 0.4. When taken as groups they rise to 0.5 to 0.6. In the groups we have not only the individual correlation but an additional amount due to the fact that these groups of dams and their daughters for the most part spend their lives on the same farm and, therefore, under very similar environments. Lush and his co-workers at Iowa have shown that there is a correlation of about 0.20 to 0.30 between cows kept in the same barn regardless of relationship.<sup>2</sup>

TABLE 3  
Group daughter-dam comparisons for butterfat test

	Number bulls	Daughter-dam comparisons	Mean test	Standard deviation	Correlation	Regression of daughters on dams
Ayrshire .....	73	540	Dams ..... 4.05 Daus. .... 4.16	0.177 0.194	0.466 ± 0.091	0.510
Brown Swiss	109	808	Dams ..... 3.99 Daus. .... 4.04	0.222 0.232		
Guernsey .....	225	1,754	Dams ..... 4.83 Daus. .... 4.91	0.256 0.262	0.446 ± 0.053	0.456
Holstein .....	561	4,635	Dams ..... 3.51 Daus. .... 3.55	0.181 0.205		
Jersey .....	234	1,656	Dams ..... 5.23 Daus. .... 5.31	0.340 0.336	0.610 ± 0.041	0.602 Av. 0.545

<sup>2</sup> JOURNAL OF DAIRY SCIENCE, 17: 737-742; 18: 811-825; and 25: 975-982. See also pages 279-284 in "Milk Secretion" by John W. Gowen.

To test this matter of group correlations and regressions further (since it is so vital a matter in indexing dairy bulls) the writer secured a list of 1,095 indexed Ayrshire bulls from the Ayrshire Breeders' Association and used the Red Book Volume 14 of the Holstein-Friesian Association of America for data on 961 indexed Holstein bulls, using the records on a C basis (M.E.; 2 x's, 305 days approximately).

Correlation surfaces of these materials were prepared and yielded the data found in table 4.

It will be observed in table 4 that the Ayrshire mean milk is lower than it was in table 1. The probable reason for this is that the figures in table 4 go back over a period of 25 to 30 years, while those of table 1 are for the most recent Ayrshire records. And in test the figure for Ayrshire dams in table 4 is somewhat lower than for daughters or for dams and daughters

TABLE 4  
*Group daughter-dam comparisons for amount of milk and butterfat test*

	Number groups	Mean	Standard deviation	Correlation	Regression of daughters on dams
Ayrshire Milk, Dams .....	1,095	8,451	1,116	$0.571 \pm 0.020$	0.632
Daus. ....	1,095	8,203	1,236		
Holstein Milk, Dams .....	961	12,095	1,799	$0.549 \pm 0.022$	0.561
Daus. ....	961	12,051	1,839		
					Av. 0.597
Ayrshire Test, Dams .....	1,095	4.02	0.187	$0.448 \pm 0.024$	0.446
Daus. ....	1,095	4.07	0.186		
Holstein Test, Dams .....	961	3.44	0.148	$0.575 \pm 0.021$	0.691
Daus. ....	961	3.47	0.178		
					Av. 0.568

in table 1. The reason for this is that table 4 includes records made by more mature cows, test dropping slightly with advancing age. The figures of this study show the present Ayrshire breed to average 8,800 pounds of 4.04 per cent milk so these figures will be used later in this article as the centers of tables for "normal expectations."

The Holstein data are all of rather recent vintage and thus need no explanation. For indexing purposes to be discussed later, we can take the present Holstein average to be 12,000 pounds of 3.46 per cent milk and we will use these figures for the centers of the Holstein normal expectation tables.

Table 4 shows that the group regressions of daughters on dams for both amount of milk and butterfat test are about 0.5 as was also shown in the lesser amount of data in tables 2 and 3. Tables 2, 3 and 4 also show that the variability (standard deviation) is reduced by about 30 to 50 per cent (contrast with standard deviation in table 1) when the material is treated in groups as is done in indexing bulls.

The matter of regression may be easily illustrated by a graph as in figure 1.

In figure 1 we have assumed a breed with an average production of 12,000 pounds. If we graph the groups of cows from 6,000 pounds to 18,000 pounds (solid line), then the average of their daughter groups will fall about on the broken line starting at 9,000 pounds and rising to 15,000 pounds. From 6,000-pound dams we "normally expect" to get daughters at the 9,000-pound level, *i.e.*, they will regress one-half the way back up to the breed average. Likewise from 18,000-pound dams we "normally expect"

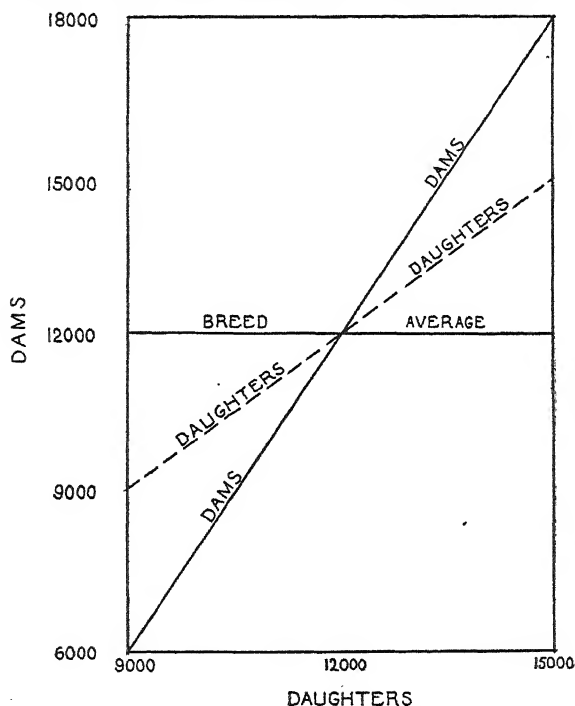


FIG. 1. Graph showing regression of 0.5 for daughters on dams. Holsteins.

their daughters to regress one-half the way back down to the breed average.

Regression, however, is not a mysterious natural law. It is due to two things: 1) the fact that phenotype does not necessarily truly mirror genotype (especially in a quality like milk production which is so prone to environmental influences) and 2) that animals producing toward the extremes are unlikely to be mated to animals as extreme as themselves.

If the breed average is 12,000 pounds of milk, we normally expect a group of daughters averaging 9,000 pounds from a group of cows averaging 6,000 pounds. Whether we would get this or not from any particular bull would remain to be seen. From a dozen bulls we might get groups of daugh-

ters all the way from 12,000 to 14,000 pounds down to 6,000 pounds, but the average of all of them would be about 9,000. There is no mysterious force, regression or any other, which will tend to move a 6,000-pound herd up to 9,000 pounds. If the owner continues to use bulls with a transmitting level of 6,000 pounds, his herd will stay at the 6,000-pound level, but if he uses average bulls it will climb up toward 9,000 pounds. Likewise an 18,000-pound herd will not automatically drop to 15,000 pounds. This herd is likely to fall back toward the breed average because its owner is likely to use average bulls. If he uses bulls with an inheritance of 18,000 pounds or more his herd will remain at 18,000 pounds or go higher.

Regression (first used by Francis Galton) is in one sense an unfortunate term because to most minds it usually connotes going backward. In the biological sense it means, according to Webster, "the correlation between parent and offspring when used as a measure of inheritance." Actually, of course, regression is the correlation times the ratio of the two standard deviations but since in much biological data the two standard deviations are approximately equal, the correlation and the regression often have approximately the same value in studies of heredity. Regression is commonly referred to as "the drag or boost of the breed." When animals get below the breed average their offspring "tend to" climb back up toward the breed average, when they get above the breed average they "tend to" fall back toward the breed average. In short, regression moves upward from below the breed average and downward from above the breed average.

Some eight or ten systems for indexing bulls have been proposed. Because of its simplicity and approximate accuracy, the one known variously as the "Equal-Parent," "Intermediate," "American" or "Yapp-Hansson" index is the one being used by the Ayrshire and Holstein breeds and the American Dairy Cattle Club (the only organizations in the United States now using bull indexes). The "Equal-Parent" index is generally calculated by finding the differences between the average amount of milk and butterfat test of a group of cows and the similar averages for their daughters by a given bull. For both amount of milk and butterfat test, if the daughters' average exceeds the dams' average, the difference is added to the daughters' average to secure the bull's "Equal-Parent" index, or if the daughters' average falls short of the dams' the difference is subtracted from the daughters' average to secure the bull's index. This system, therefore, always places the daughters just half-way (intermediate) between the dams' actual averages for milk and butterfat test, and the bull's "Equal-Parent" index for milk and butterfat test.

One form of the "Equal-Parent" index (hereafter called EP) would be:  $\text{Index} = 2d - m$  where  $d$  = daughters' average and  $m$  = dams' average.

About a dozen years ago H. W. Norton, Jr., in some unpublished work (referred to by Lush in the JOURNAL OF DAIRY SCIENCE, Vol. 16, No. 6)

suggested a use of regression of daughters on dams in computing a bull's index. His proposal was to substitute daughters' expectation (from dams of given level) for the dams' actual average production figure, and then proceed in the usual "Equal-Parent" fashion. This formula would be:  $\text{Index} = 2d - e$  where  $d$  = daughters' average and  $e$  = daughters' average expectation.

TABLE 5

*Dams' actual average and daughters' expectation centered on breed averages, regression 0.5*

Milk		Test	
Dams	Daughters' expectation	Dams	Daughters' expectation
m	e	m	e
Ayrshire			
12,000	10,400	4.60	4.32
8,900	8,850	4.08	4.06
8,875	8,837	4.07	4.05
8,850	8,825	4.06	4.05
8,825	8,812	4.05	4.04
8,800	8,800	4.04	4.04
8,775	8,787	4.03	4.04
8,750	8,775	4.02	4.03
8,725	8,762	4.01	4.03
8,700	8,750	4.00	4.02
6,000	7,400	3.60	3.82
Holstein			
16,000	14,000	4.00	3.73
12,100	12,050	3.50	3.48
12,075	12,037	3.49	3.47
12,050	12,025	3.48	3.47
12,025	12,012	3.47	3.46
12,000	12,000	3.46	3.46
11,975	11,987	3.45	3.46
11,950	11,975	3.44	3.45
11,925	11,962	3.43	3.45
11,900	11,950	3.42	3.44
8,000	10,000	3.00	3.23

The writer's proposal is to calculate a bull's index (hereafter called NEW<sup>1</sup> because it is based on Normal Expectation and breed averages which we intend to designate by  $W$ ) by finding the difference between his daughters' actual and "normally expected" productions and adding this difference to the breed average. This proposal differs from the "Equal-Parent" system in that the latter method deals with the actual records of dams and

<sup>1</sup> It might be better to ignore the potentially unfortunate connotation of the word Regression and to call this New index what is actually is viz., Regression index.

daughters without specific reference to the breed average although, as will be shown, this feature could be included. It differs from Norton's proposal in that the latter would add the difference between daughters' actual and "normally expected" productions to the daughters' actual rather than to the breed average. Differences in formulae are:

$$\begin{array}{ll} \text{EP} = d + d - m & (d = \text{daughters, } m = \text{dams}) \\ \text{Norton} = d + d - e & (e = \text{daughters' expectation}) \\ \text{NEW} = w + d - e & (w = \text{breed average}) \end{array}$$

Since the Norton index has never been used, it will not be discussed further. Its formula including the breed average would be  $w - (e - m) + 2(d - e)$ .

In practice the NEW index could be calculated from tables centered on the respective breed averages both for amount of milk and for butterfat test with daughters' expectation rising or falling from the breed average one-half as fast as their dams. Nuclei for Ayrshire and Holstein tables centered on the present respective breed average are shown as table 5.

For example if a group of Ayrshire dams averaged 12,000 pounds of milk, we would normally expect their daughters (by an average bull—regression 0.5) to average 10,400 pounds ( $e$ ). Let's suppose they actually averaged 11,600 pounds ( $d$ ) or 1,200 pounds above expectation ( $d - e$ ), then this bull's index would be,  $w + d - e$  or

Breed average	8,800	pounds
plus	1,200	pounds
	<hr/> 10,000	pounds = the bull's NEW index

If tables of normal expectation were not available, the normal expectation for any group of daughters could be ascertained by adding the respective breed averages in milk and test to the dams' average in milk and test and dividing by 2.

Similarly an optional manner of finding a bull's NEW index would be to find his EP index in the usual fashion, then add the respective breed averages to this EP index and divide by 2. In other words, the NEW method simply regresses the EP indexes half-way back to the respective breed averages.

It is the writer's opinion that a bull index should be

1. Sound from a genetic standpoint.
2. Easily arrived at and understandable.
3. Calculated in terms of the breed average.
4. Comparable in variability to groups of animals rather than to individuals.

and that it should *do* the following things:

5. Rank bulls in their proper order.

6. Provide a definite measuring stick for the bull's transmitting performance.
7. Provide a means for predicting future daughters' production.
8. Provide as accurate a means as possible for evaluating pedigrees.

The following discussion is a comparison of the EP and NEW systems of indexing in regard to the above 8 points.

(1) Sound from a genetic standpoint. Since the normal regressions of daughters on dams for amount of milk and for butterfat test are about 0.5 both the EP and the NEW methods are sound genetically both being based on a regression of 0.5.

(2) Easily arrived at and understandable. There would seem to be no choice between the EP and NEW systems in this regard. The NEW index can be secured either through the use of tables of normal expectations or by regressing EP indexes one-half of the way back to the breed averages as explained above. Comparable formulae are  $EP = d + d - m$ ;  $NEW = d + e - m$ .

In the EP system the index is as much above or below the daughters' averages as the dams are below or above, in other words the daughters are half-way between the two parental levels. In the NEW system the index is as much above or below the breed averages as the daughters exceed or fall short of "normal expectations."

(3) Calculated in terms of breed averages. Both the EP and the NEW index systems can be calculated in terms of breed averages. The formulae are  $EP = w + 2(d - e)$ ;  $NEW = w + d - e$ . In the writer's opinion it would be desirable for breeders to become cognizant of their respective breed averages. They would then have a definite measuring stick for their own and all other herds in the breed, so much above or below breed average. If a herd of Holsteins averaged 16,000 pounds, normal expectation would be 14,000 pounds; assume daughters averaged 15,000 pounds, breed average is 12,000 pounds, then  $w = 12,000$ ;  $d = 15,000$ ;  $e = 14,000$ ; therefore,

$$\begin{array}{ll} EP = w + 2(d - e) & \text{or } 12,000 + 2,000 = 14,000 \text{ index.} \\ NEW = w + (d - e) & \text{or } 12,000 + 1,000 = 13,000 \text{ index.} \end{array}$$

(4) Comparable in variability to average of groups of cows rather than to individual records. In the writer's opinion this would be desirable because indexes are computed from groups of cows and their daughters and because the index may be used as one limit, the group average (dams) as the other to predict the probable level of production of the resulting daughters. In pedigree evaluation, we have learned by experience to discount the extremely high or low records of cows, to reduce the variability in a general way. It seems likely that we must learn to do the same sort of thing with EP indexes. The NEW method of indexing bulls does this automatically, since it reduces the EP indexes above the breed average and increases those below the breed average, the NEW index being only one-half as far removed

in either direction from the breed average as is its corresponding EP index. To change any EP index to NEW index, we can simply add the breed averages to the former, divide by 2 and we have the latter.

The following table shows the variability of individuals, groups, EP indexes and NEW indexes for the Ayrshire and Holstein breeds.

It is seen from table 6 that the variability of the NEW index method is in keeping with that found in groups while the variability of the EP index method is in keeping with that found in individuals. Since in all indexing and use of indexes individuality is necessarily sacrificed to the group average, the writer believes the NEW method is to be preferred over the EP in this regard.

(5) Rank bulls in their proper order of production transmitting merit. It is well to bear in mind that no system of indexing can be any better than

TABLE 6  
*Standard deviations of daughters, dams, EP and NEW indexes*

	Ayrshire			Holstein		
	No.	Milk	Test	No.	Milk	Test
Individuals .....	(2,973)	Dams ..... 1,832 Daus. .... 1,846	0.322 0.317	(3,446)	3,036 3,147	0.277 0.279
Groups .....	(1,095)*	Dams ..... 1,116 Daus. .... 1,236	0.187 0.186	(961)†	1,799 1,839	0.148 0.178
EP indexes .....	(1,095)	2,108	0.377	(961)	2,986	0.301
NEW indexes .....	(1,095)	1,054	0.188	(961)	1,493	0.150

\* 29,324 individuals.

† 31,050 individuals.

the records on which it is based. If the daughters of bull A are fed and cared for on a higher plane than those of bull B and the person making the rating does not know this, or knowing it makes no allowance for it, then bull A is going to appear in a better light than bull B. The environment of both the cows and their daughters will affect the size of any bull's index. An index, in other words, is a relative, not an absolute, matter, made up of items of inheritance and items of environment. In the discussions in this paper we are of necessity assuming comparable environments.

Bulls can be ranked in a variety of ways. The writer thinks that from a production standpoint, it is best to rank Ayrshires, Brown Swiss or Milking Shorthorns in terms of 4 per cent fat corrected milk (Gaines formula—0.4 amount of milk plus 15 times total fat). The corresponding figures for the other principal dairy breeds are Holstein (3.46 per cent test), 0.435 amount of milk plus 16.33 total fat; Jersey (5.26 per cent test), 0.336 amount of milk plus 12.62 total fat; Guernsey (5 per cent test), 0.35 amount of milk plus 13 total fat.

Here are EP indexes for 3 Ayrshire bulls:



Bull A	12,740	lbs. milk	3.87	per cent	493	lbs. butterfat
Bull B	12,158	" "	4.04	" "	492	" "
Bull C	11,545	" "	4.47	" "	516	" "

In amount of milk they rank A, B, C; in test they rank C, A, B; in total butterfat they rank C, A, B. A consensus of these three rankings would also place them C, A, B. The 4 per cent fat corrected milk of the above three EP indexes are:

A 12,491                      C 12,358                      B 12,255

and the same figures for the NEW indexes are:

A 10,698                      C 10,564                      B 10,537

The EP and NEW systems will rank any given series of bulls in the same order since both systems are based on a regression of 0.5. EP indexes above the breed average (as in the example just cited) will be reduced, those below the breed average will be increased by the NEW method since the variability of the NEW method is just half as great as that of the EP.

(6) Provide a definite measuring stick for a bull's transmitting performance.

In this respect the NEW index method would seem to have two distinct advantages over the EP method. In the first place the NEW index provides an immediate and clear-cut evaluation of any bull's production-transmitting ability in terms of the respective breed average. A Holstein NEW index of 14,000 pounds of 3.66 per cent tells one at once that this bull's daughters exceeded "normal expectations" by 2,000 pounds and 0.2 per cent since 14,000 pounds, 3.66 per cent test is 2,000 pounds and 0.2 per cent test above the present breed average of 12,000 pounds, 3.46 per cent test. And secondly, the NEW method avoids a possible ambiguity present in any system based on a comparison of dams' and daughters' actual production. The EP method compares dams' and daughters' actual production so that if in Holsteins the dams averaged 8,000 pounds and the daughters 9,000, the bull gets a rating of 1,000 plus, and an EP index of 10,000 pounds. But from 8,000-pound Holstein dams, we normally expect 10,000-pound daughters so that this bull failed by 1,000 pounds to get his daughters up to normal expectations. Compared to an average Holstein bull, his rating is 1,000 minus and his NEW index is 11,000 pounds. In the NEW method which compares daughters' actual with their normally expected production, there should be no ambiguity in the pluses and minuses secured since the comparison tells directly whether the bull is better or worse than an average bull and how much better or worse.

Stating the difference between daughters and dams as simple pluses or minuses does not provide a necessarily accurate description of any bull's breeding worth because there is no standard point of reference. Since the normal expectation for daughters from dams below the breed average

is upward, bulls used in low producing herds generally show an increase of daughters over dams, these bulls thereby getting a plus or positive rating in dam-daughter comparisons. On the other hand, the normal expectation from dams above the breed average is downward so that bulls used in high herds generally show a decrease in their daughters, these bulls thereby getting a minus or negative rating in dam-daughter comparisons. If many breeders focused major attention on just the increases or decreases of daughters compared to dams, they would generally be favoring all bulls used or proved in low producing herds and rejecting bulls used or proved in high producing herds. If this happened the breed (and its breeders) would be harmed. Actually  $\frac{1}{2}$  the bulls used in low producing herds and  $\frac{1}{2}$  of those used in high producing herds should be favored and the other half of each rejected. Most of the bulls used in low producing herds will show an increase of daughters over dams, but only that half of the bulls whose daughters exceed normal expectations should be favored. Most of the bulls used in high producing herds will show a decrease of daughters compared with dams, but the half of these bulls whose daughters exceed normal expectations should be favored and only the other half rejected. In terms of indexes, Holstein bulls with indexes below 12,000 pounds of 3.46 per cent milk should be rejected and those above these figures favored. The amount by which a Holstein bull's NEW index exceeds 12,000 pounds of milk and a 3.46 per cent test measures directly his merit from a production standpoint. Comparable figures for Ayrshires are 8,800 pounds of 4.04 per cent milk.

(7) Provide a means for predicting future daughters' production.

The writer made a brief study of the predicting value of indexes when used in conjunction with the average production level of the bull's mates. In order to do this, 103 Ayrshire bulls were selected at random. All of these bulls had 15 or more daughter-dam pairs, they averaged 35 daughter-dam pairs. Indexes for each of these bulls were calculated on the basis of their first 10 daughter-dam pairs. For each bull this index was used in conjunction with the average production of his later mates in order to provide a prediction for the later daughters. The prediction could then be compared with these later daughters' actual average production. Since both the EP system and the NEW system are based on a regression of 0.5, the misses of prediction from actual were the same on the average, and for these data the average errors were about 700 pounds of milk and 0.12 per cent in test with the ranges running from 0 to 2,400 pounds of milk and 0 to 0.45 per cent test.

The Prediction formulae are:

0.5 EP Index + 0.5 Dams' average Production.

NEW Index + 0.5 Dams' - 0.5 Breed averages.

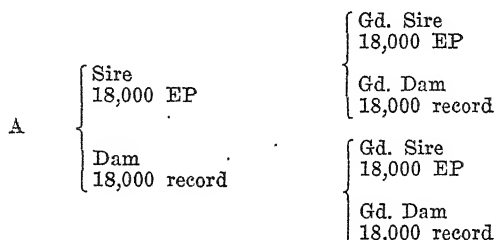
The average production of the first 10 daughters in this material gave practically as accurate a forecast of future daughters as either EP or NEW indexes used in the appropriate manner in conjunction with later mates' records. The average error using daughters alone was about 800 pounds of 0.13 per cent milk and with about the same ranges of error as found with indexes. For 86 of these 103 bulls which were used in only one herd, the average error in prediction was a little less than 700 pounds and 0.12 in test. For the remaining 17 bulls which were proved in one herd and then transferred to other herds, the average error was a little over 900 pounds and 0.14 per cent test. It would, of course, be erroneous to conclude that a bull will not transmit as well in the second herd as he has done in the first. He may transmit worse, but, of course, he may transmit better. What he appears to do will depend a lot on the environmental levels in the two herds concerned. It also will depend a lot on the genetic levels in the two herds. The genetic make-up of the cows in the second herd may not be as good a "fit" for the bull as was that of the cows in the first herd. On the other hand, of course, it may be better.

We should not confuse the matter of predicting future daughters with that of evaluating bulls. Daughters alone will predict future daughters, especially if the future daughters are to be produced in the same herd and out of much the same kind of dams, but daughters alone will not distinguish between or among bulls because the level of the dams' production does influence their daughters' production. We might have two groups of Ayrshire daughters both averaging 9,200 pounds of milk, but the sires of these 2 groups of daughters are not necessarily similar in their breeding powers. If bull A's mates averaged 7,000 pounds, we would expect their daughters to average half-way up to 8,800 or at the 7,900 pound level—they actually averaged 9,200, so bull A is 1,300 pounds better than an average bull in milk transmitting ability. If bull B's mates averaged 11,000 pounds, we would expect the daughters to average 9,900 pounds, but they too did 9,200 pounds, so bull B is 700 pounds below an average bull. These 2 bulls differ by 2,000 pounds of milk although their respective daughters' averages were the same.

(8) Provide as accurate a mean as possible for evaluating pedigrees.

The problem of evaluating the probable genetic worth of any animal from its pedigree is a difficult one at best, since pedigrees as currently written include only the selected direct ancestors and in most instances include only the most favorable data on these animals. Added to these man-made handicaps is the biological fact that inheritance is a halving and sampling process so that even though one knew a pedigree completely in terms of indexes and records, both for the direct and collateral relatives, he still would not know, except by testing and indexing, what sort of a sample half of each parent's inheritance the animal in question received and how they "nicked."

Since the direct ancestors are likely to have been carefully selected the cows' records and the bull's indexes are likely to be above the breed average in perhaps most instances. Experience has taught us to discount in a general way the extreme variations from the average which we find in the records of the cows in any pedigree. It seems probable that we would also have to learn in time to discount somewhat the EP indexes. If, for example, we had a Holstein pedigree like the following schematic pedigree what deductions would we be justified in making about genetic production level of Animal A?



Encountering the figure 18,000 all through this pedigree has a tendency to make one think that this animal must have an inheritance at about that level. However, it seems doubtful that we would be justified in ascribing an inheritance of 18,000 to Animal A although, of course, chance may have provided this or possibly even more. We now know that these 18,000-pound EP indexes indicate that these bulls' daughters produced 3,000 pounds more than normally expected since 18,000 is 6,000 above the Holstein breed average of 12,000. The more cautious among us would feel that we should at least moderate the cow's record in the schematic pedigree shown.

In terms of the NEW index these 18,000-pound EP indexes become 15,000, which has the advantage of informing us that the daughters of these bulls produced 3,000 pounds more than normally expected at whatever level their dams may have been. We can, if we like, also regress the cows' records through which they also become 15,000. In the writer's opinion, the regressed cows' records and the NEW indexes of 15,000 pounds is a more probable estimate of the genetic worth of Animal A than that likely to be derived from the actual records and the EP indexes. At least it might be a welcome change to err occasionally on the safe side.

#### SUMMARY

Individual regression of daughters on dams in dairy cattle is of the order of about 0.3 for amount of milk and 0.45 for butterfat test.

Group regressions are of the order of 0.5 to 0.6 both in amount of milk and butterfat test.

The "Equal-Parent" index is based on a regression of 0.5 for milk and butterfat test, but this fact is perhaps not generally appreciated,

nor are the respective breed averages generally used in computing EP indexes. The "Equal-Parent" index formula using normal expectation and breed average is  $w + 2(d - e)$  where  $w$  = breed average,  $d$  = daughters' average,  $e$  = daughters' expectation =  $\frac{w + \text{dams' ave.}}{2}$ .

A NEW method of indexing, based on normal expectation and breed average, is suggested herein, its formula being  $w + d - e$ .

The NEW method compares daughters' actual production with their normal expectation rather than with their dams' actual production.

The variability of NEW indexes is of the same order as is that of groups of cows.

The variability of EP indexes is of the same order as is that of individual cows.

Either EP or NEW index method will place bulls in the same order of rank from highest to lowest.

The NEW index makes it possible to ascertain a bull's worth from a production standpoint by comparing the index directly with the respective breed averages, so much better or worse than breed average, through its incorporation of "normal expectation" as a measuring stick of any bull's transmitting performance.

Either the EP or NEW index system used in the appropriate manner with the average record of a group of cows will predict the production of future daughters reasonably accurately and perhaps a little more accurately on the average than will the average of an earlier group of daughters. Daughters' records alone, however, are not a safe guide to the genetic level of a bull or at least not as good as indexing, which automatically includes a consideration of the mates' level of production; an important item so far as their daughters' ability is concerned.

The NEW method of indexing would appear to make pedigrees look more nearly like what they are genetically than does the EP method. It would provide a measure of conservatism so often and so badly needed in pedigree writing and interpretation.

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# THE OPTIMUM EMPHASIS ON DAMS' RECORDS WHEN PROVING DAIRY SIRES\*

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## INTRODUCTION

Nearly all of the proposals for expressing numerically the transmitting ability of a dairy sire are special forms of the general equation:

$$I = a + c(X - bY) \quad (1)$$

Where  $I$  = the index or measure used for comparing one sire with another.

$a$  = a constant which brings the average of the whole group of indexes to the desired level but does not alter the difference between any two sires.

$c$  = a constant which can be used to expand or contract the variability of  $I$  without changing any correlation between it and other variables.

$X$  = the average record of the daughters of the sire.

$Y$  = the average record of the dams of those daughters.

$b$  = a constant which determines the relative emphasis on  $Y$  as compared with  $X$ .

When only the average of the daughters is used as the proof of a sire, equation (1) becomes:  $I = X$ ; *i.e.*,  $a$  and  $b$  are each zero while  $c$  is 1.0. At the other extreme when the sire proof is considered to be simply the increase or decrease of his daughters over their dams,  $a$  is zero but  $b$  and  $c$  are each 1.0, whence  $I = X - Y$ . The most widely used sire index (known by various names, such as intermediate, equal-parent, modified Mount Hope, etc.) sets  $a$  equal to zero but  $b$  to 0.5 and  $c$  to 2.0; *i.e.*,  $I = 2(X - 0.5Y)$ . The recent proposal<sup>1</sup> by V. A. Rice of a "NEW" index is simply to let  $c = 1.0$ ,  $b = 0.5$ , and  $a = b$  times the breed average; whence  $I = 0.5(\text{breed average}) + X - 0.5Y$ . Turner long ago (page 24 in Missouri Research Bulletin 79 in 1925) proposed to let  $I = \frac{100}{85}(X - 0.15Y)$ ; *i.e.*,  $a = \text{zero}$ ,  $b = 0.15$ , and  $c = \frac{100}{85}$ . These examples show what diverse kinds of indexes are all included as special cases of equation (1).

The real accuracy of an index is measured by its correlation with the true transmitting ability ( $G$ ) of the sire for which it is computed. The amount of improvement made in the offspring by selecting bulls with equal intensity, but according to  $I_1$ , to  $I_2$ , . . . or to  $I_n$ , is strictly in proportion to  $r_{GI_1}$ ,  $r_{GI_2}$ , . . . or  $r_{GI_n}$ . The size of  $b$  affects  $r_{IG}$  but  $a$  and  $c$  do not.

The object of the present paper is to show what value of  $b$  will make  $r_{GI}$  as large as is possible for any index of the type described by equation (1). Also some related problems of using an index are discussed. These ideas

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<sup>1</sup> See preceding paper, this issue.

and findings arose largely as a result of discussions with Professor V. A. Rice about his "NEW" index. Helpful suggestions from him and the use of his data for reference are gratefully acknowledged, but he is not to be held responsible for the conclusions or interpretations in the present article.

### PREDICTING G FROM Y AND X

Perhaps the simplest derivation of (1) is the ordinary multiple regression equation for predicting G from Y and X. The path coefficient diagram for that, and the pertinent formulas for the best possible prediction of G from X and Y jointly, are shown on the left in figure 1.

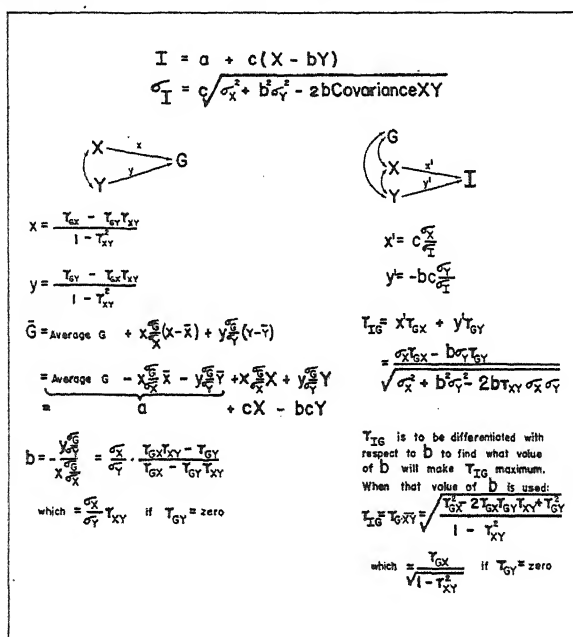


FIG. 1. Biometric relations between daughter average (X), average of mates (Y), and breeding value (G) of sire. Left: Predicting G from X and Y. Right: Correlation between G and any index (I) which is the sum or difference of any multiple of X and any multiple of Y with or without the addition or subtraction of any constant.

If  $r_{GY}$  is zero, the formula for  $b$  simplifies greatly to:  $r_{XY} \frac{\sigma_X}{\sigma_Y}$ , . . . , i.e., to  $\frac{\text{Covariance } XY}{\text{Variance } Y}$ . That is, the optimum value of  $b$  is the regression of X on Y, as Rice maintains. If  $r_{GY}$  is a small positive, the optimum value of  $b$  will be somewhat less, as is shown more clearly by rewriting the formula for  $b$  (figure 1) as follows:

$$b = \frac{\sigma_X}{\sigma_Y} \left[ \frac{r_{XY} - \frac{r_{GY}(1 - r_{XY}^2)}{r_{GX} - r_{GY} r_{XY}}}{1 - r_{XY}^2} \right]$$

The last term within the brackets goes to zero when  $r_{GY}$  does but must have



a positive value when  $r_{GY}$  does, since  $r_{GX}$  is practically certain to be considerably larger than  $r_{GY}$ .

The  $r_{GX}$  will be positive and of considerable size because each daughter gets half of her G as a sample half of her sire's G. Each daughter's record (O) is partly determined by her own G. Many of the other factors which can make her record large or small will be random from one daughter to another and hence will tend to cancel each other in the average ( $\bar{X}$ ) of several daughters. The relation between  $r_{GX}$  and  $r_{GO}$  is as follows:

$$r_{GX} = r_{GO} \sqrt{\frac{n}{1 + (n-1)w}}$$

where there are  $n$  daughters and the correlation between the records of paternal sisters is  $w$ . In most dairy data collected from many different herds but analyzed as a single population,  $w$  is around 0.2 to 0.3, much of this coming from environmental differences between herds, although  $w$  also includes  $r_{GO}^2$ . Hence in most data used for proving dairy sires  $r_{GX}$  will be something like 1.5 to 2.0 times as large as  $r_{GO}$ . Reasonable values for  $r_{GO}$  (approximately half the square root of the heritability of differences between individual cows) in most dairy data are around 0.2 to 0.3 for quantity of milk or fat and around 0.3 to 0.4 for test.

For  $r_{GY}$  to have a positive value requires that there be a general tendency for the breeders who already have high producing cows to try harder than average, and for the breeders who have cows with low records not to try as hard, to get good bulls to mate with them. Further, such a difference in efforts would produce a positive  $r_{GY}$  only to the extent that the breeders estimate correctly the breeding values of the bulls at the time of choosing. Presumably there is some difference of this kind in the efforts made but this gives  $r_{GY}$  only a very small positive value because the correlation between the real transmitting ability of an untried young bull and the purchaser's estimate of that from the bull's pedigree, or from other information available when the bull is first put to work, is generally small. We shall not be far wrong if we proceed on the assumption that  $r_{GX}$  is much larger than  $r_{GY}$  although the latter may not be quite as low as zero.

How a positive correlation between G and Y could lower the proper value of  $b$  is readily understandable when one reflects that if those breeders whose herd averages are already high do generally succeed in buying young bulls with better-than-average breeding value, then a man seeking to find the best young bulls will have some degree of success if he does nothing but choose the bulls being used in herds which already had better-than-average production at the time those bulls were introduced. This is the line of thought we follow when we sometimes infer that a young bull bought for use in a high-producing herd is probably an exceptionally good bull or he would not have been selected for use in that herd. (Of course we are often wrong in such an inference, but there may well be a gambler's margin in favor of

it.) Under such conditions Y becomes a positive indicator of G in its own right and not merely a negative indicator, useful for discounting the effects of environmental differences from herd to herd and the effects which genetic differences between groups of mates have on the records of their daughters, which is its usefulness when  $r_{GY}$  is zero.

#### MAXIMIZING THE CORRELATION BETWEEN I AND G

The optimum value of b can also be found, by setting up the equation for  $r_{GI}$  as indicated on the right side of figure 1, differentiating it with respect to b, and then finding what value of b will make that differential equal to zero. That value of b turns out to be the same as is shown on the left side of figure 1, as of course it should be. The two ways of finding the optimum value of b are the same in principle.

#### ACTUAL VALUES FOUND FOR b

The values found by Rice for the regression of X on Y for milk in seven sets of data ranged only from 0.49 to 0.70 with an unweighted average of 0.60. For fat test the same sets of data yielded values ranging from 0.45 to 0.69 with an average of 0.55. Using 0.5 for b in dairy data will be nearly correct, especially if  $r_{GY}$  has some small positive value. On page 33 of the report of the New Zealand Dairy Board for 1943 a table of expectations for fat production indicates that the regression of X on Y (the proper value of b if  $r_{GY}$  is zero) in those data is about 0.58 to 0.62. This table is based on 20,150 daughter-dam pairs which were used in proving 1395 sires. Parenthetically it may be noted that, since the observed regressions are a little larger than 0.5, the daughter-dam difference seems just a shade more accurate as a sire index than the daughter average alone.

#### GAIN FROM CONSIDERING THE RECORDS OF THE MATES

The amount of improvement made in the next generation by selecting on the basis of I is  $\frac{r_{GI}}{r_{GX}}$  times the improvement to be made by selecting (with the

same intensity) for X alone. If  $r_{GY}$  is zero, this factor reduces to  $\frac{1}{\sqrt{1-r^2_{XY}}}$  which in Rice's data has values ranging from 1.14 to 1.27, with an unweighted average of 1.21 for milk, and from 1.12 to 1.26 with an unweighted average of 1.18 for test. Making some rough allowance for  $r_{GY}$  having a small positive value and for the fact that in actual practice the value used for b will not always be the exact optimum value for that particular set of data, the use of I would make improvement from sire selection something like 12 to 20 per cent faster than if X were used alone. Thus the gain from using properly the records of the mates along with the records of the daughters when proving a sire is not extremely large in dairy data, although it certainly is real.

It is sometimes argued that the gain from including the mates is not enough to balance the loss from excluding those daughters which are out of untested dams. For this to be true would require that there be few daughters out of tested dams, that there be many out of untested dams, and that the correlation between the records of daughters of the same bull be low. If this latter correlation is as low as 0.24 and bulls are selected on the records of their daughters alone, progress will be 11 per cent faster when they have ten daughters than when they have five; if the correlation between daughters is  $+0.30$ , the corresponding gain will be 9 per cent instead of 11, while with a correlation of  $+0.36$  it will be increased only 7 per cent, and with a correlation of  $+0.42$  it will be increased only 6 per cent. The above correlations are about what exist between paternal sisters in various aspects of Rice's data on Ayrshires and Holsteins. Therefore the loss from omitting entirely the daughters from untested cows would rarely equal the gain to be had by considering Y properly, even if that required omitting half the daughters. Moreover, where the record of a mate is missing, one could substitute almost as well in the index the average of the other mates or (better still) the average of that daughter's contemporary herd mates who are not by the same sire. With the spread of herd testing, as contrasted with testing only selected individuals, the proportion of daughters who are out of untested dams becomes ever smaller, more of the mates having been tested themselves as daughters in the proving of some earlier sire.

It thus appears that almost the only cost of getting the extra 12 to 20 per cent of progress to be had by including the records of the mates is the clerical cost of assembling and computing their records.

#### CAUSES OF THE CORRELATION BETWEEN X AND Y

The correlation between the records of individual daughter and dam has generally been reported as of the order of  $+0.3$  to  $+0.4$  in most studies of data collected from many different farms but analyzed as a single population. Why such a correlation will usually be different from the  $r_{xy}$  which describes the data as they actually are *grouped* in the proving of sires is explained as follows. Figure 2 shows, in terms of path coefficients, how  $r_{xy}$  is constituted. The letters have the following meanings:

O = the record of a daughter.

D = the record of a mate.

r = the correlation between the record of a daughter and the record of her own dam.

v = the correlation between the record of a daughter and the record of a mate of her sire other than her own dam.

u = the correlation between the records of two mates of the same sire.

w = the correlation between the records of two daughters of the same sire.

In the numerator of  $r_{xy}$  (formula shown in figure 2) v occurs  $n-1$  times as often as r. The denominator starts out as 1.0 when  $n=1$  but, as  $n$  becomes

indefinitely large, this denominator tends toward  $n$  times the geometric mean of  $u$  and  $w$ , *i.e.*, toward  $n\sqrt{uw}$ . It seems simplest to think of  $r_{XY}$  as a complex average consisting roughly of one part  $r$  and  $n-1$  parts  $\frac{v}{\sqrt{uw}}$ . Most of this shift of  $r_{XY}$  from  $r$  toward an expression which is mainly dominated by  $v$ ,  $u$ , and  $w$ , is usually accomplished by the time  $n$  is as large as five. The regression of  $X$  on  $Y$  is  $\frac{\sigma_O}{\sigma_D} r$  when  $n = 1$  but tends toward  $\frac{\sigma_O}{\sigma_D} \cdot \frac{v}{u}$  as  $n$  becomes indefinitely large.

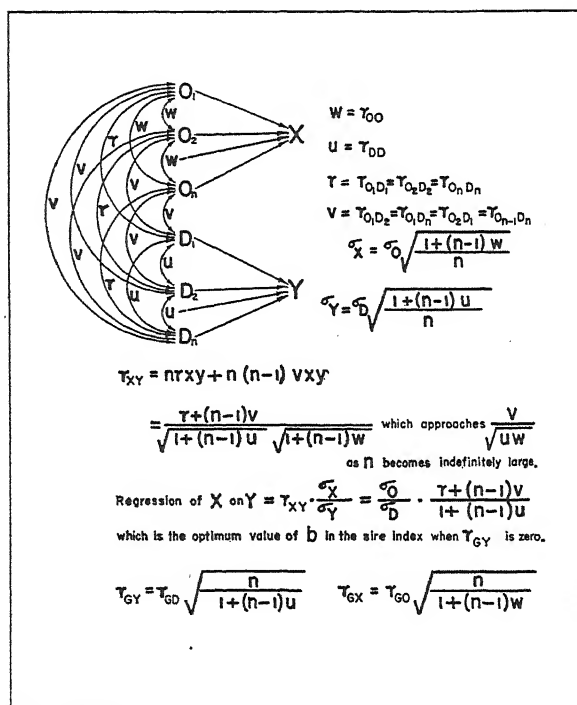


FIG. 2. Biometric relations between  $X$  and  $Y$ , showing how  $r_{XY}$  is constituted of  $r$ ,  $v$ ,  $u$ , and  $w$ , and may be very different from  $r$ .

In proving dairy sires,  $n$  has a minimum value of five (in the Dairy Bureau procedure—six, ten, or more in various of the registry association procedures), but is usually variable in any list of proved sires from which  $r_{XY}$  may be calculated. That is, sires which have been proved on exactly five daughter-dam pairs will be included in the same list with sires which have been proved on six, seven, or more pairs. Variations in  $n$  cause  $r_{XY}$  or  $b$  or  $\sigma_X^2$  or  $\sigma_Y^2$  to shift from one limiting value toward another in proportion to the changes in the reciprocal of  $n$ . Therefore, the harmonic mean<sup>2</sup> of  $n$

<sup>2</sup> The harmonic mean of  $n$  is the  $n$  which has a reciprocal equal to the mean of the reciprocals of the actual  $n$ 's.

should be used when analyzing into their constituent parts ( $r$ ,  $v$ ,  $u$ , and  $w$ ) the  $r_{xy}$ ,  $b$ ,  $\sigma_x$  or  $\sigma_y$  computed on a population of proven sires in which  $n$  was a variable. The harmonic mean will be less—sometimes considerably less—than the arithmetic mean. For example, the first 220 indexed sires in Volume 14 of the Holstein-Friesian Red Book had  $n$ 's varying from 6 to 83, although for half of them  $n$  was 12 or less. The arithmetic mean was 15.2 while the harmonic mean was 11.2. For the first 152 Holstein proved sires in Misc. Pub. 522 from the U. S. Dept. of Agr. the arithmetic mean was 8.77 but the harmonic mean was 7.60. Here  $n$  ranged from five to 35 but half the sires were proved on seven pairs or less. Among the 73 Ayrshire proved sires listed in the same publication, the arithmetic mean was 7.40 and the harmonic mean was 6.88.

In table 1 are shown the values for  $u$ ,  $w$ , and  $v$  computed from the data Rice shows in his tables 1 and 4. Because the statistics on  $X$  and  $Y$  (Rice's

TABLE 1

*The ingredients of  $r_{xy}$  in T. A. Rice's data on Holsteins and Ayrshires*

Statistic	Milk		Test	
	Ayrshire	Holstein	Ayrshire	Holstein
$r^1$	0.29	0.32	0.48	0.43
$v^2$	0.26 <sup>5</sup>	0.18	0.12	0.18
$u^3$	0.36 <sup>5</sup>	0.28	0.28	0.22
$w^4$	0.47 <sup>5</sup>	0.28	0.28	0.36
$2(r-v)$	0.06 <sup>6</sup>	0.29	0.72	0.51

<sup>1</sup> As given by Rice.

<sup>2</sup> Computed from the values of  $r_{xy}$  in Rice's table 4, substituting the values given for  $r$  in his table 1 and the values computed here for  $u$  and  $w$ .

<sup>3</sup> Computed from the formula:  $\frac{\sigma_y^2}{\sigma_D^2} = \frac{1+(n-1)u}{n}$ .

<sup>4</sup> Computed from the formula:  $\frac{\sigma_x^2}{\sigma_O^2} = \frac{1+(n-1)w}{n}$ .

<sup>5</sup> Somewhat inflated because the data in Rice's table 4 include a considerable time trend.

<sup>6</sup> The correct figure here will be larger than this by about twice the size of the bias mentioned in footnote 5.

table 4) came from a slightly different sample of Herd Improvement Registry data than the statistics on  $O$  and  $D$  and because in dairy data the

standard deviation tends to vary with the mean, Rice's observed  $\frac{\sigma_y}{\sigma_D}$  and  $\frac{\sigma_x}{\sigma_O}$

were multiplied by the ratio of the corresponding means before computing  $u$  and  $w$ . Even after this correction, the figures for  $u$ ,  $w$ , and  $v$  are still inflated (as compared with  $r$  which comes wholly from Rice's table 1) wherever the data from Rice's table 4 covered a longer period in which there was a marked time trend. This seems to have been important only for the Ayrshire milk. The data concerning Ayrshires in table 4 were collected over a period of 25 or 30 years, whereas the Holstein data go scarcely half that far

back. For Ayrshire milk the time trend was marked, since the means in Rice's table 1 are 7.1 per cent larger for daughters and 5.8 per cent larger for dams than the corresponding means in his table 4. For Ayrshire test and for the Holstein data the time trend was too small to have much effect on the present analysis, the maximum increase of any of these means in his table 1 over the corresponding mean in his table 4 being only 1.2 per cent. For computing the  $u$ ,  $w$ , and  $v$  shown here in table 1, the observed harmonic mean of 11.2 was used for  $n$  in the Holstein data and 11.5 was used in the Ayrshire data. (The Ayrshire arithmetic mean actually was 13.39 but the distribution of  $n$  was not available for computing the harmonic mean directly.)

### Causes of $r$ , $u$ , $v$ , and $w$

Figure 3 shows how  $r$ ,  $u$ ,  $w$ , and  $v$  are caused partly by differences in breeding value and partly by differences in environment. Environment is used here to include all other causes of variation in the records except differences in the genetic value of the cows which made them.

The meaning of the symbols is as follows:

$g^2$  is the fraction of the phenotypic variance ( $\sigma_o^2$  or  $\sigma_D^2$ ) due to additively genetic differences between individual cows.

$e^2$  is the fraction of the phenotypic variance which is not additively genetic.

$d$  is the genetic correlation between mates of a sire. It has a moderate positive value because some of the mates are related to each other and also because some breeders try harder than others to breed and select for high production.

$m$  is the average genetic correlation between the sire and a mate. It will be very little above zero, since most breeders try to avoid even mild inbreeding. Assortive mating on somatic likeness must be indirect since the male cannot exhibit the characteristic himself. Hence assortive mating can contribute but little to  $m$ . Moreover assortive mating is not extreme for these characteristics, since no one tries intentionally to mate low producing cows to bulls with unusually low production in their pedigrees.

$r_{EE}$  is the correlation between the non-genetic causes of variation for the individual denoted by the subscripts. It has a moderately large positive value because herds differ much from each other in their management, as well as in uncontrolled environmental conditions such as weather, condition of pastures, etc. Generally  $r_{EE}$  will be larger between daughters than between mates, or than between a daughter and a mate, because the daughters' records are more nearly contemporaneous and thus are subject to more nearly the same peculiarities of management and weather or other environment.

$r_{GE}$  exists only to the extent that the herds with the highest intrinsic breeding values are also fed and managed better than the average herd, while herds with low intrinsic breeding values are fed and managed less well than average. There may well be some of this in dairy data but  $r_{GE}$  must be small because of the uncertainty concerning the breeding value of the average animal while it is yet alive and because no one intentionally tries to collect and breed low producers. The  $G$  and the  $E$  in  $r_{GE}$  pertain to different individuals.

The environmental terms and the terms for cross-correlations between genetic and environmental causes of variation (figure 3) are almost the same

in  $r$  as they are in  $v$ , except when a sire is proved in two or more herds. In such cases the environmental term included in  $r$  is certain to be larger than the corresponding one in  $v$ . Because their other terms are so nearly the same, subtracting  $v$  from  $r$  leaves a remainder which comes close to equalling  $\frac{g^2(1-d)}{2}$ . Doubling this and then making allowance for  $d$  yields an estimate of the heritability of differences between records of mates of the same sire. Figures for  $2(r-v)$  are shown in the bottom line of table 1. Dividing these by something like 0.85 to 0.95 (to allow a reasonable amount for  $d$ )

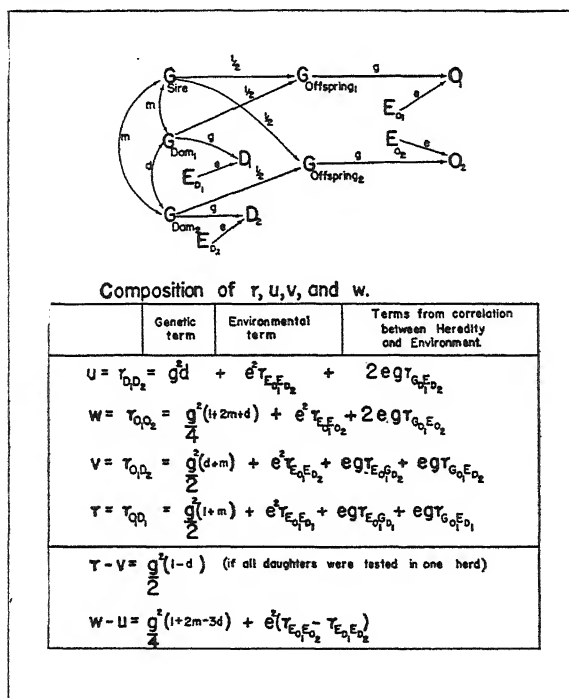


FIG. 3. Path coefficient diagram showing the causes of the correlations between the records of daughters and of dams.

yields estimate of heritability of intra-breed differences between cows. A small amount should then be deducted from that to allow for the (comparatively few) cases in which a sire was proved in two or more herds differing (of course) somewhat in management. The estimate of milk in the Ayrshire breed is certainly too low because of the time trend which contributed considerably more to  $u$ ,  $w$ , and  $v$  than it did to  $r$ ,<sup>3</sup> as was mentioned above. The

<sup>3</sup> The figure for  $r$  came wholly from Rice's table 1 which covered only a short period of time. The figures for  $u$ ,  $w$ , and  $v$  came from differences or ratios between statistics in his table 1 and in his table 4, the latter having extended over a considerably longer period.

three other estimates are compatible with most of those derived from other studies, namely something under 0.3 (perhaps under 0.2) for differences in milk records and something of the order of 0.5 to 0.7 (or less if the data included many sires which each had daughters in more than one herd) for differences in test. A strong influence of contemporaneity on Ayrshire milk and Holstein test may be indicated by  $w$  being so distinctly larger than  $u$ , but perhaps that needs confirmation on more extensive data before effort is spent on finding an explanation for it. Certainly the daughters' records would generally have been made within a more restricted range of time than the records of the dams.

The similarity of  $r_{XY}$  in the data for Herd Improvement Registry (Rice's table 4) and in the Dairy Bureau data for Dairy Herd Improvement Associations (Rice's tables 2 and 3) tempts one to suppose that heritability and the other factors affecting sire proving are the same in both kinds of data. However, the numerical value of  $r_{XY}$  depends more on the ratio of  $v$  to  $u$  and  $w$  than it does on the difference between  $r$  and  $v$ . The topic merits further study.

#### OPTIMUM VARIABILITY FOR SIRE INDEXES

The variability of a sire index does not alter its *accuracy* (provided there are at least 16 to 20 classes from the lowest to the highest figure) but may affect considerably its convenience in use and its susceptibility to misinterpretation. The variability of the index can be made as large or as small as one chooses by altering the value of  $c$ . Two plausible definitions for the variability which a sire index should have for maximum convenience in actual use are as follows: A. The index should equal the most probable breeding value of the sire. B. The index should have the same standard deviation as the records of cows.

The theoretical advantages of standard A are obvious. It expresses the sire proof directly in terms of the goal for which indexes (and indeed all forms of progeny testing or estimating breeding value) are intended. Under it the  $c$  of equation (1) should make  $c_I = r_{GI}c_G$ . To do this requires that  $c$  be approximately  $\frac{g^2}{2} \cdot \frac{c_0^2}{c_X^2} \cdot \frac{1}{1 - r_{XY}}$  which in the data pertaining to dairy sires comes close to  $2g^2$ . For example, it has values of 1.65, 1.82, 2.10, and 1.84  $g^2$  in Rice's table 6 for Ayrshire milk and test and Holstein milk and test, respectively. Unfortunately the value of  $g^2$  is not known with high certainty (*i.e.*, within really narrow fiducial limits) in any population. In most dairy data it seems to be around 0.15 to 0.30 for quantity of milk and somewhat higher—perhaps above 0.50—for test. Moreover  $g^2$  will vary a bit as there is more or less care in controlling or correcting for environmental variables, a larger or smaller number of daughters, whether single records or lifetime averages are used for daughters and for dams, etc.

Attempting to state the proper numerical value for  $c$  turns the spotlight



on the practical difficulty of using standard A, namely the uncertainty about the precise value of  $g^2$  to use, the variation (usually slight) in that from one population to another, and the large variation in  $g^2$  from one characteristic to another, with the resultant necessity of using one formula for test, another for milk, etc. Also many will think "most probable breeding value" is more theoretical and intangible than an actual record (D or O) which is a familiar and very real thing to them. If standard A is used, the index of a sire cannot be compared directly with the record of a cow until the cow's record is first translated to the scale of "probable breeding values" by regressing it  $1 - g^2$  of the way toward the breed average.

Any change from one standard to another will always cause considerable confusion. It would be unfortunate to adopt standard A now and start indexing sires, using 0.40 as  $c$  for milk and 0.80 as  $c$  for test, only to find three or four years later that 0.50 and 1.10 or 0.30 and 0.70 would have been more accurate for most dairy populations. Eventually we may come to standard A or something very similar, but the change should first receive considerable scrutiny and much trial and actual practice.

Standard A automatically discounts the records for the average amount of non-genetic variation in them and thus protects the user against too easily falling a victim to his wishful thinking. No index can be guaranteed to show the breeding values of each individual proven sire correctly but a scale which is just as likely to rate an individual too low as too high is less susceptible to misinterpretation than one on which the high indexes are generally higher and the low indexes are generally lower than the breeding values of the sires to which they apply.

Standard B puts the indexes of sires and the records of dams on an equal footing, so that they can be compared directly.<sup>4</sup> For  $\sigma_I$  to equal  $\sigma_D$  exactly

<sup>4</sup> Strictly speaking, this requires that mean I and mean D be approximately equal (since in most dairy data  $\bar{X}$  differs little from  $\bar{Y}$ ) and that  $\frac{\sigma_I}{r_{IG}} = \frac{\sigma_D}{r_{DGd}}$  where  $Gd$  is the breeding value of the cow who has record D. The writer has shown (JOUR. DAIRY SCI., 18: 1-19, 1935) that  $r_{IG}$  and  $r_{IGd}$  will not be far apart if heritability is larger than 0.10 and especially if (as seems almost always to be the case in dairy data collected from several herds) the environmental contribution to the correlation between daughters of a sire is +0.10 or larger. The argument may be reviewed here in slightly different terms by referring to the composition of  $r_{IG}$  as shown in figure 1. When  $r_{Gx} = \text{zero}$  and  $r_{xx} = 0.5$ ,  $r_{IG}$  reduces to  $r_{Go} \sqrt{\frac{n}{1+(n-1)u} \cdot \frac{4}{3}}$ . Now  $r_{Go}$  is half the correlation between a daughter's record and her own breeding value. This latter correlation will be the same as between a dam's record and her breeding value ( $r_{DGd}$ ), except as more intense selection among the dams than among the daughters may have reduced  $r_{DGd}$  slightly and more lactations per dam than per daughter may have raised  $r_{DGd}$ . Hence  $\frac{r_{IG}}{r_{DGd}}$  is approximately  $\frac{\sigma_o}{\sigma_x} \cdot \frac{1}{\sqrt{3}}$  which isn't very far from unity. It has values ranging from 0.86 to 0.99 in Rice's table 6 but perhaps should be increased a little to allow for the dams having been a bit more highly selected than the daughters were. In short, selection of dams on their own records will rarely be either much less or much more accurate than selection of sires on their indexes.

requires that  $c = \frac{c_D}{\sqrt{c_X^2 + b^2 c_Y^2 - 2b r_{XY} c_X c_Y}}$ . If  $r_{GY}$  is zero and  $r_{XY} = 0.5$ , this

is  $\sqrt{\frac{4}{3}} \sqrt{\frac{n}{1+(n-1)w}}$  which, for most of the likely values of  $n$  and  $w$ , gives  $c$  a value not far from 2.0—more often a little less than a little more. Rice's table 6 would require for  $c$  values of 1.74, 1.71, 2.03, and 1.85, respectively, to make the index conform exactly to standard B. The EP index, now rather widely used, has 2.0 for  $c$  and therefore comes fairly close to standard B. The EP index cannot be equalled for simplicity of computation, among indexes which use an approximately correct value for  $b$ .

If the index is used only for comparing the sires with each other and with cows, no further step than standard B is necessary. For reducing indexes (or records of cows) to breeding values or for predicting the production of future offspring the indexes (or records) need to be regressed toward the average of the breed far enough ( $1 - g^2$  of the way) to allow for the average amount of non-genetic variance in them. If this second step is neglected or not understood, the user of indexes constructed according to standard B (or the user of cows' records) may easily build hopes too high (in terms of actual pounds or per cent) on the bulls or cows with the high figures and may damn more severely than he should those with low figures.

In principle standards A and B differ only in that the regression toward the breed average is "built into" the operation of figuring the index under A and hence is already accomplished when the index is obtained, while B requires two steps to reach the same goal. The first step yields the index itself, which can be compared directly with the records of cows but is more variable than breeding values. The second step (which is not necessary for comparing sires with each other or with cows and hence is often omitted) is to estimate probable breeding value from the index by regressing it  $1 - g^2$  of the way toward the breed average.

Rice's NEW index, which uses 1.0 for  $c$ , comes near to standard A for test but the proper value for  $c$  for quantity of milk is not that large. The breeding values of sires for amount of milk or of fat will generally be nearer to the breed average than their NEW indexes. The NEW indexes are simply EP indexes regressed half way toward the breed average—*i.e.*, the NEW index for each bull is exactly half way between his EP index and whatever constant figure is used for the breed average in computing the NEW index. Since this difference is only one of coding (*i.e.*, the EP index is divided by two and then has a constant added to it to form the NEW index), the two indexes have the same correlation with any other variable and are equally accurate for comparing one sire with another.

#### PREDICTING THE ACTUAL PRODUCTION OF FUTURE DAUGHTERS

For predicting the production of a future daughter the correct procedure in principle is simply to average the most probable breeding values of the dam

and the sire. Then this average should be raised or lowered enough to allow for the environment in which the daughter is to make her record being better or worse than average. But in practice we rarely have any direct measure of that environment. Probably the average record of that herd for the most recent two or three years could be used advantageously for this purpose, but this is not yet being done generally. The environment pertaining to the daughter will usually be correlated slightly (something of the order of  $+0.2$  to  $+0.3$ ) with the record of her dam if she and her dam make their records in the same herd. It will usually be still more closely correlated (something of the order of  $+0.4$  to  $+0.6$ ) with the index of her sire if she is to make her record in the same herd as the one in which her older sisters made the records on which that index is based. In this latter case the sire index assumes a large part of the predictive value which would attach to the direct measure of the daughter's herd environment if such a measure were available. Because of its lower correlation with the herd environment, the dam's record does not assume much of this predictive value for environment if the sire index was made in the same herd but it does assume considerable if the daughter is to make her record in the same herd as her dam but in a different herd from that in which her sire was proved. If the daughter is to make her record in a herd in which neither her dam nor her sire's earlier daughters were tested (an uncommon case in dairy data), then both the sire's index and the dam's records are useful only for their genetic relation and neither of them will help as an indicator of the herd environment under which the daughter will make her record.

If sire indexes are to be used almost solely for estimating the production of future daughters, either singly or in groups, then one can make a good case for building into the index enough extra variation to allow also for its importance as an indicator of non-genetic circumstances (*i.e.*, for the kind of environment) which will prevail for that daughter or those daughters. But  $D$  also should receive extra weight for its real, although generally lesser, usefulness for the same thing. This leads at once to four different scales for  $c_1$  and  $c_D$  (or four different factors by which to multiply them), according to whether the future daughter is to make her record in the same herd as her dam and her older paternal sisters, in the same herd as her dam but a different one from her sisters, in a different herd from her dam but the same herd as her sisters, or in a different herd from either her dam or her sisters. Possibly there is some simple way of doing that but it seems to the writer probable that the simplest way will be to use standard A (which is standard B regressed  $1 - g^2$  of the way toward the breed average) and then modify the prediction up or down according to whether the most recent average of the herd in which the daughters are to be tested is above or below the breed average.

Predictions of the production of an individual future daughter cannot be expected to be highly accurate. A correlation of around 0.1 to 0.2—*i.e.*,

$g^2$  times the square root of one-half—between the actual and the predicted record of the individual daughter (for quantity of fat or milk—a bit higher for test) is about as much as can reasonably be expected if the dam has only one lactation and the sire's proof was of only average accuracy and the daughter is to make her record in a different herd. Although this would be raised distinctly if the dam is judged by all of her records and if there were three or more of those, and if the sire's proof is unusually accurate by reason of a large number of daughters and unusually careful discounting of the environmental circumstances which applied to his daughters and mates, yet it seems unduly optimistic to expect by that means to attain an average accuracy as high as a correlation of  $+0.4$  between actual record and predicted record.

The average production of  $n$  future daughters can of course be predicted more accurately than the production of one daughter. This is only an automatic result of the averaging process and does not introduce any new biological principle. The averaging permits many of the chance circumstances, which cause a daughter to produce more or less than was predicted, to cancel each other's effects in the average of  $n$  daughters. The correlation between prediction and fact is  $\sqrt{\frac{n}{1+(n-1)w}}$  times as large when predicting the average production of  $n$  daughters as when predicting the production of one daughter. Also  $\sigma_x$  is only  $\sqrt{\frac{1+(n-1)w}{n}}$  as large as  $\sigma_0$  and this of itself makes the error of prediction seem smaller, if that error is measured in actual pounds or percent instead of being measured relative to  $\sigma_x$  or  $\sigma_0$ . The net result is that in actual units the standard error of estimating the average of  $n$  daughters is only  $\sqrt{\frac{1-w+n(w-t^2)}{n(1-t^2)}}$  as large as the standard error of estimating one daughter, where  $t$  is the correlation between prediction and fact when predicting one daughter.

#### SUMMARY

Nearly all sire indexes which have been proposed can be described by the general equation,  $I = a + c(X - bY)$ , in which  $a$ ,  $b$ , and  $c$  are constants,  $X$  is the average production of the daughters,  $Y$  is the average production of their dams and  $I$  is the index.

The size of  $a$  affects only the general level (the mean) of the indexes. The size of  $c$  affects the variability of  $I$  but not its accuracy for comparing the breeding values ( $G$ ) of two or more indexed sires. The size of  $b$  affects the accuracy of the index as well as its variability.

The main contribution of this paper is in showing that maximum accuracy of the index is attained when  $b = \frac{\sigma_x}{\sigma_y} \cdot \frac{r_{GX}r_{XY} - r_{GY}}{r_{GX} - r_{GY}r_{XY}}$ . If  $r_{GY}$  = zero this

optimum value of  $b$  becomes simply the regression of  $X$  on  $Y$ . If  $r_{GY}$  has a small positive value (as is possible if breeders whose cows have high records generally try harder than other breeders to get good bulls—and if the extra efforts are partially successful) the optimum value of  $b$  is a little less than the regression of  $X$  on  $Y$ . The regression of  $X$  on  $Y$  is about 0.5 to 0.6 both for milk and for test in most sets of data actually used for proving dairy sires. The optimum value for  $b$  in dairy data will, therefore, be not far from 0.5.

If  $r_{GY}$  is zero, selection of sires on the optimum index, as thus defined, will make  $\frac{1}{\sqrt{1-r^2_{XY}}}$  times as much progress as choosing the sires on the average of their daughters alone. The size of this factor, when  $r_{GY}$  is very small and  $r_{XY}$  has such values as are usually encountered in proving dairy sires, is about 1.12 to 1.20.

The size of  $r_{XY}$  or of the regression of  $X$  on  $Y$  is affected more by the correlation ( $v$ ) between a daughter's record and the record of a mate of her sire, other than her own dam, than it is by the correlation ( $r$ ) between a daughter and her own dam, especially when  $n$  is large. The regression of  $X$  on  $Y$  approaches  $\frac{v}{u}$  and  $r_{XY}$  approaches  $\frac{v}{\sqrt{uw}}$  as a limit when  $n$  becomes extremely large,  $u$  being the phenotypic correlation between the mates of the same sire and  $w$  being the phenotypic correlation between daughters of a sire.

A sire index can be made as variable as desired by adjusting  $c$ . The value 2.0, used for  $c$  in the intermediate or equal-parent indexes makes  $c_1$  generally just a little larger than  $c_D$  or  $c_0$ . This index can be used rather fairly for comparing proven sires directly with individual cows, as is necessary in evaluating pedigrees. It is, however, more variable than real breeding values. Consequently, if it is to be used directly as the sire's most probable breeding value, the index needs first to be regressed far toward the breed average (just as cows' records do) to allow for the average amount of non-genetic variation in such indexes. Approximately this amount of regression would already be accomplished in an index which used for  $c$  twice the heritability of differences between the records of individual cows. Rice's proposed "NEW" index, which uses 1.0 for  $c$ , is the equal-parent index regressed half way toward the breed average. It is, therefore, half as variable but has exactly the same accuracy.



# THE RELATIVE CONCENTRATIONS OF DAM AND FETAL LIVER GLYCOGEN\*

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The composition of the blood of calves immediately following birth differs in certain respects from that of their dams. It was reported by Knodt, Shaw, and White (3) that the blood acetone bodies of calves at birth are significantly lower than that of their dams. Bodden and Allcroft (1) presented data demonstrating higher levels of blood sugar, serum calcium, inorganic phosphorus, and non-protein nitrogen but a lower level of chlorine in the blood of calves as compared to their dams at the time of parturition. Because of the relationship which apparently exists between the calves and their dams relative to the concentrations of blood glucose and acetone bodies, it was decided to determine the levels of liver glycogen of feti and their dams and compare these observations with those previously made.

## EXPERIMENTAL

Previous work has shown that the level of blood glucose of calves at birth is higher than that of their dams. It would appear that a somewhat similar relationship might exist with regard to the concentrations of liver glycogen. A high level of liver glycogen in calves should be important in the maintenance of a higher concentration of blood glucose and a lower level of blood acetone bodies in calves following birth as compared to their dams. It is obvious that data relative to the concentrations of liver glycogen are difficult to obtain and involve the destruction of the animals. The animals used in this work were those passing through a local abattoir.

The age of the feti was estimated as 8 to 9 months. The cows were killed in the usual manner and the samples of the left lobe of the dam and fetal livers were removed immediately for glycogen analyses. The tissue samples were placed in warm, previously weighed 50 per cent potassium hydroxide and the glycogen analyses were made by the method of Good, Kramer, and Somogyi (2). Data are presented in table 1 on 20 cows and their feti. The average concentration of fetal liver glycogen was 4.12 per cent whereas that of the dams averaged 0.25 per cent. In no case was the level of fetal liver glycogen lower than that of the dam. The level of fetal liver glycogen varied from 2.67 per cent to 5.36 per cent while that of the dams varied only from 0.15 per cent to 0.38 per cent.

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The low level of glycogen in the dams' livers was probably due in part to the effect of inanition since these cows had probably not been fed for at least 24 hours previous to slaughter. It is significant that the feti were able to maintain a comparatively high level of liver glycogen in spite of the extremely low level of liver glycogen of their dams.

A somewhat similar relation may exist in normal cows where these levels may not differ so markedly. When these observations are related to the comparative levels of blood acetone bodies of calves and their dams at the time of parturition (3) it will be observed that there is an inverse relation-

TABLE 1  
*Concentration of glycogen in dam and fetal livers*

Expt. No.	Concentration of liver glycogen (%)	
	Fetus	Dam
1	4.50	0.20
2	3.85	0.24
3	3.29	0.22
4	5.41	0.27
5	3.48	0.21
6	3.88	0.26
7	5.06	0.35
8	4.23	0.17
9	2.96	0.28
10	3.52	0.26
11	4.22	0.38
12	4.76	0.31
13	5.02	0.18
14	4.90	0.27
15	5.36	0.23
16	4.16	0.23
17	4.40	0.34
18	3.86	0.18
19	2.67	0.26
20	2.93	0.15
Average	4.02	0.25

ship between the levels of liver glycogen and the level of blood acetone bodies. It has been observed that the level of acetone bodies is higher in the dam than in the calf at the time of parturition. It has been shown that the feti maintain a higher liver glycogen under the conditions of the experiments. This is in direct agreement with the observations of Bodden and Allcroft (1) demonstrating the higher blood glucose level of calves as compared to their dams.

Observations have been presented by Roderick, Harshfield, and Hawn (4) in which it was shown that the liver of a new-born lamb possesses a glycogen reserve of 2 to 3 times that of the liver of the ewe. They performed biopsies and removed portions of the livers of ewes and lambs which showed evidence of ketosis of starvation and showed that abnormal deposition of fat had occurred in these livers. Evidence was also presented to show that



upon feeding these animals until they had returned to normal the fat had been gradually removed from the liver and glycogen had been stored.

Samples were taken from the livers of 15 calves from 3 to 6 weeks of age. The age was again estimated. The samples were taken from the left lobe of the livers immediately after the calves were killed but showed considerable variation in the concentration of liver glycogen. The average of these samples was 2.60 per cent with a range from 0.24 per cent to 7.81 per cent glycogen.

It appears that the levels of various compounds such as liver glycogen, blood glucose and acetone bodies, serum calcium, inorganic phosphorus, non-protein nitrogen, and chlorine are maintained at levels in the feti which are to a certain extent independent of the levels concurrently present in the dam.

#### CONCLUSIONS

1. The fetus is able to maintain a much higher level of liver glycogen than that of its dam under the particular conditions encountered in these experiments.
2. There appears to be an inverse relationship between the concentrations of liver glycogen and blood glucose as compared with acetone bodies between the fetus and its dam at the time of and prior to parturition.

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# THE STIMULATION AND INHIBITION OF MILK SECRETION IN GOATS WITH DIETHYLSTILBESTROL<sup>1, 2</sup>

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Research work of the past few years has suggested the rather anomalous fact that the administration of estrogens (and diethylstilbestrol) in certain amounts may be effective in stimulating the initiation of milk secretion in virgin or parous non-lactating animals while at the same time established lactation may be seriously depressed by these same hormones. Folley *et al.* (4, 5, 6), Lewis and Turner (8, 9, 10, 11), Walker and Stanley (25), and Reece (20, 21) have adequately demonstrated that diethylstilbestrol, a synthetic estrogen, will initiate lactation in dairy goats and cattle. Many investigators (see Meites and Turner (14) for review) using the smaller experimental animals have claimed that estrogen can diminish or completely suppress lactation and, in fact, was the mechanism by which lactation was held in check during pregnancy (24). In goats de Fremery (7) reported depression of lactation with estrogen and in cattle Folley (2) and Walker and Stanley (25) observed similar effects. These latter workers suggested that large amounts of estrogen inhibit while smaller amounts stimulate milk secretion.

If lactation is inhibited by large amounts of estrogen (or diethylstilbestrol) the mechanism of this inhibition should be sought since Meites and Turner (14) showed that diethylstilbestrol even in relatively huge doses increased the lactogenic hormone content of the pituitary of rats and guinea pigs. Further, if the use of diethylstilbestrol is to become a practical procedure for the initiation of lactation in sterile animals, the dosages which will stimulate lactation in various species must be clearly differentiated from those which will suppress lactation.

## PROCEDURE

The goats used in these experiments were grade Toggenburgs. They were fed a mixed grain ration and either lespedeza or alfalfa hay and were milked twice daily. During the experimental periods diethylstilbestrol dissolved in olive oil was injected subcutaneously once daily or was administered orally by capsule.

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## EXPERIMENTAL RESULTS

*Initiation of lactation.* The first section of this paper is concerned with the initiation of lactation in five virgin yearling goats by the injection of 0.25 mg. per day of diethylstilbestrol, a dosage which was found by Lewis and Turner (11) to be optimal for this purpose in goats. Injections in these goats (Nos. 258, 351, 176, 355 and 569) were started on September 8, 1942, and were continued until January 9, 1943. Injections were reinitiated on February 8, 1943, and were continued for nearly another month at which time the experiment was terminated by taking a mammary gland from each animal for histological study. Milking of these animals was started on December 9, 1942, and was continued through the balance of the experiment.

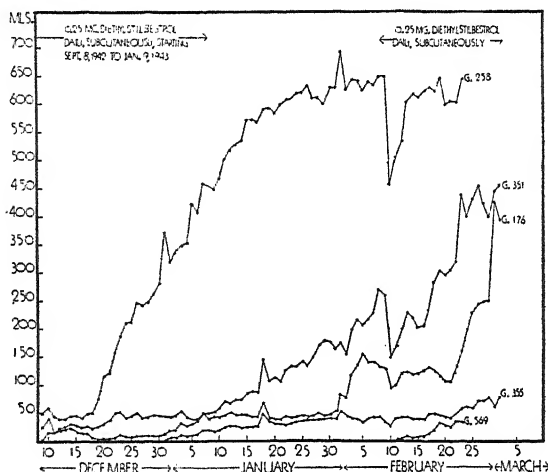


FIG. 1. The milk production records of five virgin female goats in which lactation was induced by the daily subcutaneous injection of 0.25 mg. of diethylstilbestrol. Injections were started on September 8, 1942, and were continued through January 9, 1943, were reinitiated on February 8, 1943, and were continued through the balance of the experiment.

As may be seen in figure 1, there was a great variation in the amount of milk given daily by the individual goats under identical treatment. It is interesting to note that there was no special reaction in milk yield when the diethylstilbestrol was discontinued on January 9, 1943. However, when the administration of diethylstilbestrol was reinitiated on February 8, 1943, there was an immediate drop in milk production in all goats. This drop was quickly recovered and in two instances (Goats 351 and 176) the milk production which followed was considerably higher than that previously attained. It will be noted in the case of Goat 569 that milk secretion was not initiated at all until after the start of the second period of diethylstilbestrol administration.

A report on the state of mammary gland growth in these animals at the end of the experiment (February 24, 1943, for Goats 258 and 569, and March 3, 1943, for Goats 351, 176, and 355) has already been made (18). This study of the mammary glands indicated that milk production was roughly proportional to the amount of mammary gland growth secured. The highest producing goat (No. 258) had a rather extensive development of the lobule-alveolar or milk secreting system, while the low producing goat (No. 569) showed only a hypertrophy of the duct system with limited milk secretion. It would seem, then, that individual goats vary widely in their lactation response to diethylstilbestrol, primarily because of the great difference in mammary growth elicited by the standard dosage of diethylstilbestrol. Of course, it is also possible that the lactogenic hormone of the anterior pituitary was not stimulated to the same degree in the various animals by the diethylstilbestrol.

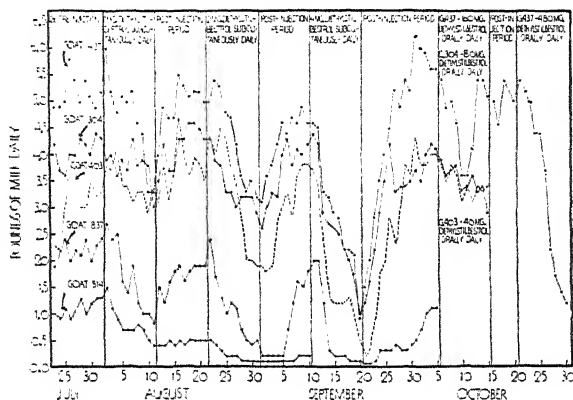


FIG. 2. A record of milk production and diethylstilbestrol administration in five parous goats. Note the depression in milk production coincident with the injection of 1.0 mg., 2.0 mg. and 4.0 mg. of diethylstilbestrol daily for ten-day periods, respectively. Also note the depression in milk production starting October 6, following the oral administration of diethylstilbestrol.

*Inhibition of lactation.* The second section of this paper is concerned primarily with the effect of varying dosages of diethylstilbestrol on established lactation in parous goats. The usual procedure was to inject the diethylstilbestrol daily over a ten-day period and then allow a ten-day post-injection period. By the trend of milk production over these periods the effect of the diethylstilbestrol injections could be determined.

In preliminary trials three goats were used to determine the effect of daily injections of 0.1 mg. and 0.2 mg. of diethylstilbestrol on milk production. No effects on milk production ascribable to the diethylstilbestrol could be detected. In two other goats the effects of 1.0 mg., 2.0 mg., and 4.0 mg. of diethylstilbestrol daily on milk production was studied. The indi-

cations from these two goats were that 1.0 mg. of diethylstilbestrol daily inhibited milk production slightly while 2.0 and 4.0 mg. dosages of diethylstilbestrol daily were very definitely inhibitory in nature. However, these goats were in an advanced stage of lactation and the results may have been influenced to some degree by this fact.

Therefore, in later trials five goats were used in similar studies. Three of these goats (Nos. 437, 304 and 403) were in early stages of lactation while the two other goats (Nos. 837 and 514) were in more advanced stages. Ten-day periods of diethylstilbestrol injections were followed by ten-day periods in which no material was injected. The daily dosage of diethylstilbestrol during the first injection period was 1.0 mg., followed in subsequent injection periods by 2.0 and 4.0 mg. daily. The course of these injections and the milk production records of the goats are presented in figure 2. From this figure it will be noted that the lactation is inhibited in all cases by the dosage of diethylstilbestrol injected, the degree of inhibition being proportional to the dosage. Recovery in milk production was effected in all cases in which the original level of milk production was relatively high (Goats 437, 304 and 403).

#### RELATION OF ORAL TO SUBCUTANEOUS ADMINISTRATION OF DIETHYLSTILBESTROL

The oral administration of diethylstilbestrol in small experimental animals and in man has been quite effective in comparison with subcutaneous administration. In ruminants, however, there appears to be large losses in the digestive tract. Folley *et al.* (4) gave 1 gm. as a drench to a cow without effect on lactation. Lewis and Turner (11) observed that 5 mg. per day orally was less effective than 0.25 mg. per day injected subcutaneously in stimulating lactation in goats. Zondek and Sulman (26) have observed that many types of microorganisms inactivate diethylstilbestrol. It appears likely that the microorganisms in the rumen may be responsible for the relative ineffectiveness of this hormone when given orally.

The rather uniform depression of milk secretion in Goats 437, 304 and 403 with increasing dosages of diethylstilbestrol (fig. 2) presents a method for determining the relative activity of diethylstilbestrol administered orally as compared to that subcutaneously administered. Thus on October 6, 1943, oral administration of diethylstilbestrol was initiated, Goat 437 receiving 160 mg. daily, Goat 304 receiving 80 mg. daily and Goat 403 receiving 40 mg. daily for a ten-day period (fig. 2). The lower dosages were rather ineffective or only slightly effective in inhibiting lactation while the highest dosage was slightly effective. In a subsequent period, Goat 437 received 480 mg. of diethylstilbestrol orally daily. The suppression of lactation secured with 480 mg. of diethylstilbestrol orally roughly approximated that secured in the same goat with 4.0 mg. administered subcu-

taneously. These results would indicate then that diethylstilbestrol is roughly 1 per cent as active in inhibiting lactation when administered orally to goats as compared to subcutaneous administration. Presumably, this same relationship would be true for lactation-stimulating dosages. Thus if 0.25 mg. of diethylstilbestrol subcutaneously is effective in stimulating lactation, 25 mg. would be required to be equally effective orally.

#### DISCUSSION

The experiments with goats reported in this paper serve to distinguish between a lactation-stimulating dosage and a lactation-inhibiting dosage of diethylstilbestrol. The variability in the response of goats on the lactation-stimulating (0.25 mg) level raises the question whether a slightly higher dosage might have stimulated greater mammary gland growth and lactation. It is also possible that 0.5 to 1.0 mg. per day might stimulate more extensive growth of the gland even at the expense of lactation and that upon the reduction of the level to 0.25 mg., the lactation-stimulating response would predominate.

The data presented confirm and extend the earlier observations of Folley (2, 3) Walker and Stanley (25) and Lewis and Turner (11) that certain levels of diethylstilbestrol will depress established lactation. The present observations indicate that the inhibition is temporary and lactation may actually return to as high or higher level following the period of administration, at least in early stages of lactation.

The large oral requirement of diethylstilbestrol in comparison to the subcutaneous dosage points to huge losses in the rumen or other parts of the digestive tract. This is unfortunate as diethylstilbestrol might otherwise be added to the grain mixture of sterile animals to initiate the lactation process.

Meites and Turner (14, 15, 16, 17) suggested that lactation is initiated at about the time of parturition by the presence of increased amounts of physiologically active estrogen acting upon the pituitary and stimulating the secretion of increased amounts of the lactogenic hormone. The action of the lactation-stimulating dosage of diethylstilbestrol would fit in with this theory. The fact that large amounts of diethylstilbestrol do not inhibit the secretion of the lactogenic hormone necessitates another explanation for the lactation-inhibiting action of this compound.

It has been suggested that estrogen may inhibit milk secretion by acting directly upon the secreting cells of the mammary gland (Nelson, 19). There are several reasons to believe that the action of estrogen in producing an increased hyperemia and permeability of the vascular system of the mammary gland would be favorable to the transfer of milk precursors to the secreting epithelium (Mixner and Turner, 18).

During the past few years, it has been demonstrated that diethylstilbestrol causes enlargement of the adrenal cortex (see 1, 12, 22, 23 for

review). This effect is produced through the mediation of the pituitary and, presumably, by the stimulation of increased secretion of the adrenocorticotrophic hormone. Certain hormones secreted by the adrenal cortex are known to increase the rate of deamination of the amino acids (protein catabolism) with the production of glucose (12). If the process of deamination were to be carried on too actively, the nitrogenous precursors of milk protein would become limited and the milk secretion process might be expected to be inhibited.

It is significant that diethylstilbestrol (22, 23), adrenocorticotrophic hormone (13) and certain adrenal cortical hormones have depressing effects upon the growth process also. It is believed that both milk secretion and the growth process are depressed by the same chain of events stemming from the action of diethylstilbestrol upon the pituitary and the adrenal. Other factors which are known to cause adrenal hypertrophy would be expected similarly to depress milk secretion (23).

The adrenal hypertrophy observed in some species during late pregnancy and the return to normal size during early lactation may explain the mechanism of control of the rising segment of the lactation curve. Pathological conditions at the time of parturition, such as retained placenta, which would maintain adrenal hypertrophy would inhibit milk secretion so long as the condition persisted.

#### SUMMARY

The relation between lactation-stimulating and lactation-inhibiting dosages of diethylstilbestrol in dairy goats was established in these experiments. A daily dose of 0.25 mg. of diethylstilbestrol constitutes a lactation-stimulating dosage in nonparous goats while dosages varying from 1 to 4 mg. per day were progressively lactation-inhibiting in lactating goats.

As judged by the lactation-inhibiting effect, diethylstilbestrol orally administered is only about 1 per cent as effective as when administered subcutaneously.

The lactation-stimulating effects of small dosages of diethylstilbestrol are due to its ability to stimulate the secretion of the lactogenic hormone by the anterior pituitary gland while the lactation-inhibiting effects are believed to be correlated with increased adrenal-cortical activity resulting in an increased rate of deamination of the nitrogenous precursors of milk protein. It is suggested that the hormones of the adrenal cortex play a role in regulating the course of the rising segment of the lactation curve.

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# THE CONTROL OF DIARRHEA ("WHITE SCOURS") OF NEW-BORN DAIRY CALVES BY MEANS OF SERUM AND SULFAGUANIDINE\*

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Wherever cattle raising is an intensive industry, diseases of various types frequently occur in new-born calves, losses of which sometime reach appalling magnitudes in individual herds. The most prevalent cause of these losses (13, 15, 16, 17) is "white scours."

The control of this disease is of great economic importance to the dairy-men in their expansion program and of interest to the nation in its nutritional readjustments. Prior to the rapid advance in chemoprophylaxis and chemotherapy, satisfactory means of coping with this problem were not available. Recent observations on the use of several of the sulfa compounds in the control of calf diarrhea are promising from a practical viewpoint (10, 29, 31, 35, 36, 37).

The primary purpose of this investigation was to obtain additional clinical information on the relative values of a commercial anti-scour serum and one of the sulfonamides, sulfaguanidine (sulfanilylguanidine), as agents for controlling diarrhea of new-born calves under practical herd conditions.

## LITERATURE REVIEW

General information on the subject of "white scours" is summarized by Marshall (19), Simms *et al.* (23), Van Es (33) and others. Early experimental investigations pertaining to this and similar diseases of calves have been adequately reviewed and presented by Hagan (8), Hart and Traum (12) and Pfenninger (21).

*Nature of "white scours."* This disease, also known by various other descriptive terms, is ill defined symptomologically and etiologically. "White scours," according to Williams *et al.* (34), generally refers to an acute diarrhea occurring from a few hours to several days after the birth of the calf. Although scours manifested in an older calf is regarded as a different type and is designated by other terms, the line of demarcation is not clear. Chronological differentiation seems to be adequate to distinguish "white scours" from diarrhea of parasitic origin but not from that of nutritional etiology. Scours due to a deficiency of vitamin A (11) and/or

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to members of the B-complex (22) is occasionally confused with the "white" type. Investigators further agree that this malady frequently coexists with or merges into not only different types of diarrhea but also other diseases, particularly pneumonia, in such a manner that symptomatic delineation is difficult.

Even though past investigations indicate that "white scours" is a diarrhea of bacterial etiology, the possibility that the implicated micro-organisms may be secondary invaders cannot be disregarded (2, 9, 14). Organisms of the colon group generally are agreed to be the predominating type present, but no investigator holds that any one organism or variety alone is the all-important etiological factor involved.

The bacterial invasion, according to a summary by Hart and Traum (12), usually takes place post-natally but occasionally *in utero*. The most important channel of infection is the digestive tract, but the umbilical vessels also may be a path of invasion.

*Predisposing factors.* The predisposing causes of "white scours" are numerous but may be grouped in the following classes: congenital weakness, improper calf husbandry and inadequate nutrition. General recognition of the importance of these first two factors obviates further comment, but recent developments in the nutritional aspects of calf diarrhea warrant consideration in the etiology of "white scours."

The composition and properties of colostrum offer a clue to the physiological needs of the new-born calf. Early investigations by Smith and his associates (24, 25) demonstrated the importance of colostrum in protecting calves against generalized infections by *B. coli*. The protective characteristic has been ascribed to antibodies, but this does not preclude the possibility that the vitamins of the colostrum may play either complementary or supplementary immunological rôles. Latterly, cumulative fragments of evidence tend to support this conjecture.

Investigations by Dann (5) revealed that colostrum is the primary medium through which vitamin A is transmitted to the new-born calf. The immunological importance of the amount of this vitamin in the colostrum milk is suggested by the report of Stewart and McCallum (27) showing that calves from dams secreting colostrum having less than 250 blue units per 100 ml. were more susceptible to "white scours" and associated ills than were calves from dams having a greater concentration of vitamin A activity in the colostrum. Recent observations by Moore and Berry (20) indicated further that whenever the colostrum ingested failed to increase the vitamin A and the carotene of the plasma of a new-born calf, it usually died from infection. Either poor quality colostrum or faulty absorption might have been involved in these cases. The relationship of the quantity of vitamin A activity in colostrum to its protective properties needs further investigation.

Phillips and associates (18, 22) emphasized the rôle not only of vitamin A but also that of members of the B-complex and ascorbic acid in the control of calf diarrhea of nutritional origin. However, Thorp (29, 30) was unable to control an acute type of scours, evidently of bacterial etiology, in the day-old calf by the use of vitamins A, B and C as prophylactics.

*Sulfaguanidine as a control agent.* The general control methods employed in protecting the new-born calf from "white scours" are sanitation, isolation, immunization and chemotherapy. The production of sulfaguanidine has resulted in rapid expansion of chemotherapy in the control of gastro-enteric infections of livestock. Since this compound is readily dissolved in the gastro-intestinal fluid but is not absorbed to any great extent through the intact intestinal mucosa, relatively high concentrations can be maintained in the lumen of the bowel without developing sufficiently high blood and systemic concentrations to produce serious toxic reactions. The bacteriostatic and bactericidal action of this drug on certain organisms markedly alters the flora of the alimentary tract (28), thus rendering the compound effective in the control of various types of calf scours.

The prophylactic dosage of sulfaguanidine for diarrhea of new-born calves has not been established. On the basis of a limited number of observations Wise (35) suggested the following dosage schedule per pound of body weight: first dose, at two to six hours after birth, 0.05 gram; second dose, six hours later, 0.04 gram; and subsequent doses, continued from one to three days of age, 0.025 gram given morning, noon and night. Though this afforded protection, more recent investigations indicate that the quantity prescribed is greater than necessary.

The therapeutic value of sulfaguanidine for "white scours" was indicated in a preliminary report by Wise and Anderson (36). Subsequently Simms and associates (23), from a study of a limited number of cases, stated that this chemical merited further tests. Later reports of extensive and detailed investigations by Thorp *et al.* (29, 31) revealed that sulfaguanidine, properly prescribed, may be used as a curative agent for scours not only of very young calves but also of older ones.

The therapeutic dosage per pound of body weight recommended by various investigators (3, 31, 35, 36) ranges from 0.13 gram to 0.18 gram for the first day, 0.07 gram to 0.17 gram for the second day, 0.07 gram to 0.12 gram for the third day, and 0.06 gram to 0.075 gram subsequently up to six days. Thorp and Shigley (31) prescribed a lower rate for calves weighing less than 75 pounds than for those weighing more, but others (3, 35, 36) suggested the same rate for calves of all sizes. The former also recommended that, throughout, the daily dosages be divided into three equal parts; whereas the latter advocated that the initial doses during the first day be greater than those following, but that the doses for subsequent days be divided into equal parts.

Thorp and Shigley (31) observed that when approximately three times the therapeutic dose was given, toxic symptoms were evidenced in calves. Bankowski and Haring (4) also noted toxic effects when four to five times the therapeutic dose was given. The principal symptoms were "depression, anorexia, hematuria and crystalluria; in the later stages ruffled hair coat, weakness of hind quarters with stiffness in gait. Death from uremic poisoning was preceded by anuria and coma."

The dosage problem is that of producing and maintaining the proper concentration of sulfaguanidine in the digestive tract until the pathogenic organisms are overcome.

#### EXPERIMENTAL

The experimental observations were made principally in the Clemson College dairy herd following an outbreak of an acute infectious and con-

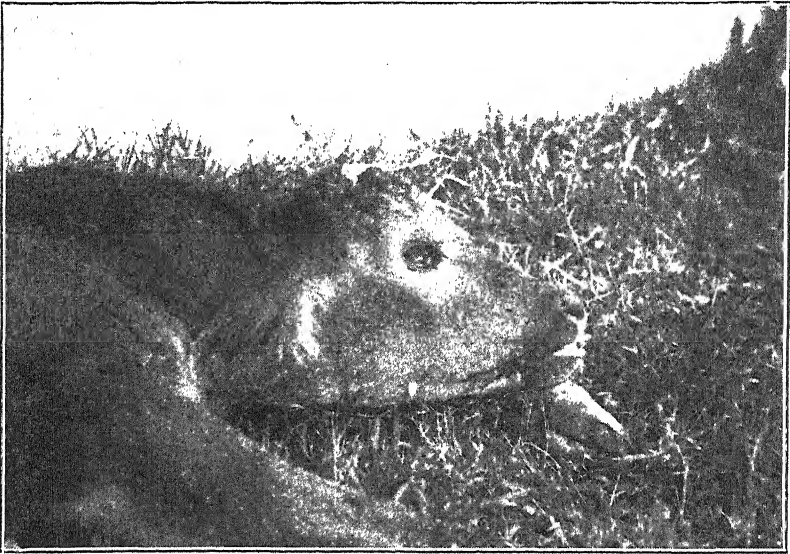


FIG. 1. Calf showing two of the characteristic symptoms, sunken eyes and profuse salivation, of "white scours" in the advanced stages.

tagious diarrhea among the new-born calves in 1940. Since then, there has been a recrudescence of the disease in spite of scrupulous sanitation.

The experimental subjects for the study of control measures included all the female calves, regardless of congenital weakness, born during the periods of investigation, but only a portion of the males since many of them were removed from the herd within a few hours after they were born.

There was a marked difference in the incidence of the disease among the various breeds (tables 2 and 4) suggesting a greater susceptibility in the Guernseys and the Jerseys than in the Holsteins.

The first symptoms of this malady generally were manifested clinically when the animals were between one and two days of age (table 5). Though the syndrome varied somewhat in individual cases, it was characterized by a variable body temperature, frequently high in the early development and subnormal in the late stages; a loss of appetite; a profuse, fetid, brown to gray serous scours; a rapid dehydration as evidenced by the eyes sinking into their sockets (figure 1) and by an increasing dryness and harshness of the skin; a progressive exhaustion, and a fatal termination within 12 to 24 hours of the onset.

A further indication of the marked dehydration in the scouring calves was hemoconcentration (table 1). Since the loss of 10 per cent of the water of the body causes serious disorders, and the loss of twice this amount causes death (7), it is probable that the severe dehydration is the physiological change primarily responsible for the rapid fatal exhaustion.

TABLE 1  
*Typical changes in concentration of hemoglobin in blood of new-born calves dying of acute diarrhea*

No. of calf	Amount of hemoglobin per 100 cc. blood		Increase in concentration of Hb.
	Within 6 hr. after birth	Within 6 hr. before death	
	<i>gram</i>	<i>gram</i>	<i>per cent</i>
G-170	11.6	14.4	24.1
A-147	10.7	16.4	53.3
499	10.5	16.1	53.5
Ave.	10.9	15.6	43.1

The autopsy findings also were variable depending on the severity and duration of the disease. Gastro-enteritis was present in all cases, some of which were complicated by septicemic conditions, especially the protracted cases. Hyperemia of the mucosa of the abomasum, of the small intestine and of a short section of the anterior portion of the large intestine was one of the most common abnormalities. Frequently the mucous membrane of the abomasum and the small intestine also was edematous and covered with scattered areas of petechial and confluent hemorrhages. The abomasal contents ranged in volume from one to three or four liters and in consistency from fluid entirely to a combination of fluid and lumps of undigested curd. The size of this coagulated mass apparently was related to the amount of colostrum milk consumed during the early stages of the disease. The bowel contents were similar to the excrement in appearance, consistency and odor. Whenever the disease reached the septicemic stage, other organs, particularly the lungs and the heart, were involved.

The bacteria isolated from the organs of four of the calves were primarily of the coliform group, which is the type most frequently found by other investigators.

## CONTROL MEASURES

The control measures investigated included in addition to general herd hygienics, the use of vitamins A and D (cod liver oil), a commercial polyvalent anti-scour serum and sulfonamides.

*Cod liver oil.* In view of the fact that vitamin A deficiency is considered a factor predisposing calves to diarrhea, a preliminary approach to the problem was made by giving cod liver oil (Nopco XX)<sup>2</sup> in doses of 20 to 25 cc. as a prophylactic agent, morning and night. This treatment was initiated when the calf was six to 12 hours of age and continued until it either contracted scours, in which case the dosage was doubled as a therapeutic, or until the calf was three days of age. Since cod liver oil was relatively ineffective in the limited number of cases observed, this treatment was discontinued.

These results indicate that excessive amounts of vitamin A do not augment perceptibly the prophylactic value of colostrum from cows maintained under good herd management. In cases of deficiencies it is probable that vitamin supplements would be beneficial.

*Antibacterial serum.* Though reported results from the use of calf-scours serum on the market have been inconsistent (12), the successes were considered sufficient to warrant further investigation. A commercial polyvalent antibacterial serum (Anti-Coli-Enteritidis-Pasteurella Serum<sup>3</sup>) was used prophylactically and therapeutically in doses ranging from the prescribed amount (10 to 20 cc. for prevention and double this amount for treatment) up to five times these quantities in efforts to control the diarrhea. The initial prophylactic dose usually was given subcutaneously within 30 minutes after the birth of the calf, but in several cases six hours elapsed before the injection.

The results of observations on the prophylactic efficiency of the serum during a 30-month period following the outbreak of the diarrhea are presented in table 2. It is obvious from this summary that the serum did not reduce the incidence of scours in the calves. Yet, it would be fallacious to assume that the serum was deleterious as the data indicate, since it was used only when the incidence was high. The failure of the serum to immunize the calves against the causal agents may be attributed to its impotency and/or non-specificity. It is probable that the preparation and use of an homologous hyperimmune serum would have been a more effective prophylactic (6) than the commercial preparation.

The twofold use of the serum as a prophylactic at birth and as a therapeutic when the first symptoms of diarrhea were noted saved seven, 63.6 per cent, of 11 calves; whereas none of 20 untreated cases, irrespective of the use of the serum as a prophylactic alone, survived. The cost, however, of re-

<sup>2</sup> National Oil Products Co., Harrison, N. J.

<sup>3</sup> Jen-Sal Laboratories, Inc., Kansas City, Mo.



TABLE 2

*Prophylactic value of commercial polyvalent antibacterial serum in the control of acute diarrhea in new-born female dairy calves*

Breed	Antibacterial serum		No prophylactic	
	Number of calves	Contracted scours	Number of calves	Contracted scours
		<i>per cent</i>		<i>per cent</i>
Holstein	51	7.8	28	10.7
Guernsey	32	34.4	18	27.8
Jersey	44	40.9	33	15.2
Cross	5	60.0	6	0.0
All breeds	132	27.3	85	15.3

peated injections of large doses of the serum as a therapeutic rendered this measure prohibitive for practical dairy herd management.

*Sulfaguanidine (sulfanilylguanidine)*.<sup>4</sup> This compound was used both as a prophylactic and a therapeutic agent. In all cases the doses were given in an aqueous suspension as a drench.

a. *Prophylaxis*. Since the most susceptible period in the life of the calf is from birth to 48 hours, it was necessary to employ preventive agents only for this period. The dosage schedules shown in table 3 were effective in reducing the incidence in three herds where scours was prevalent among the new-born calves. In herd A, composed of Jerseys, Guernseys and Holsteins, two-gram doses were given throughout regardless of breed, size and age of calf. In herds B and C the size of the dose was decreased with age since the resistance of calves is known to increase rapidly from birth to two days.

Listlessness and anorexia were noted occasionally in calves following the initial dose. One observer<sup>5</sup> found that this could be averted by withholding the sulfaguanidine until the calf had nursed.

This observation suggests that colostrum affects the absorption of sulfaguanidine. The relationships of the age of the calf and the kind and the

TABLE 3

*Dosage schedules for sulfaguanidine given as a prophylactic for scours of new-born calves in different herds*

Dose	Age of calf	Amount of sulfaguanidine per cwt. of calf		
		Herd A (Mixed)	Herd B (Holstein)	Herd C* (Guernsey)
	<i>hours</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
1	2-6	2.2-3.6	2.1	3.7
2	8-12	2.2-3.6	1.6	2.9
3	20-24	2.2-3.6	1.0	2.2
4	32-36	2.2-3.6	.....	.....

\* Dosage rate calculated from standard weight of calves for breed.

<sup>4</sup> Supplied by Lederle Laboratories, Inc., New York, N. Y.

<sup>5</sup> E. T. McClure, Dairyman, South Carolina State Hospital Dairies, Columbia, S. C

amount of feed consumed to the absorption of this chemical and its consequent toxicity and effectiveness remain to be determined. Thorp (29) stated that in the practical use of sulfonamides cognizance should be taken of the fact that they are more toxic for younger calves than for older ones. Since the permeability of the intestinal tract to and the selective absorption of certain colostral constituents are known to be markedly reduced during the first day after birth, it is possible that age and/or colostrum affect the permeability of the intestinal mucosa to the sulfonamides. Further information on this point is needed to establish the optimum prophylactic dosage schedule for sulfaguanidine.

b. *Therapeutics.* The therapeutic effectiveness of sulfaguanidine for scours in new-born calves is indicated in tables 4 and 5. The incidence

TABLE 4

*Comparative incidence of scours in new-born female calves and the resulting mortality rate during two consecutive periods: (I) without sulfaguanidine therapy and (II) with sulfaguanidine therapy*

Breed	15-month periods	Total No. of calves	Calves contracting scours	
			Total	Mortality rate
			<i>per cent</i>	<i>per cent</i>
Holstein	I	36	2.8	0.0
	II	43	13.9	0.0
Guernsey	I	27	37.0	70.0
	II	23	26.1	16.7
Jersey	I	46	32.6	73.3
	II	31	29.0	11.1
Cross	I	5	40.0	100.0
	II	6	16.7	0.0
All breeds	I	114	23.7	74.1
	II	103	21.4	9.1

(table 4) was essentially the same for the comparative periods, before sulfaguanidine therapy was initiated and after, but the mortality rates were strikingly lower in the latter period. The 25.9 per cent survival in period I resulted from the use of the aforementioned combined serum prophylaxis and therapeutics.

Of the 56 cases treated with sulfaguanidine (table 5) 26 were females and 30 were males in which groups the respective mortality rates were 7.69 per cent and 20.0 per cent. The higher fatality in the males is attributable primarily to the extreme stages to which the disease was permitted to advance in order to ascertain its critical points. Prognosis was unfavorable, regardless of the quantity and frequency of dosing, after any of the following conditions developed: excessive intestinal hemorrhage, extreme dehydration, and subnormal body temperature.

The total amount of sulfaguanidine given and the number of doses (table 5) varied over a wide range depending upon the severity and the

TABLE 5

*Effectiveness of sulfaguanidine as a therapeutic agent for diarrhea of new-born dairy calves*

Breed	No. of cases	Ave. age symptoms first noted	Ave. wt.	Sulfaguanidine given		Mortality rate
				Ave. No. doses	Total amt./cwt.	
		<i>hours</i>	<i>lb.</i>		<i>grams</i>	<i>per cent</i>
Holstein	18	31	90	2.0	10.8	22.2
Guernsey	20	43	69	2.0	14.1	10.0
Jersey	17	38	57	2.3	11.7	11.8
Cross	1	36	70	3.0	12.2	0.0
All breeds	56	40	72	2.14	12.3	14.3
	Range	16-144*	41-124	1-6	4.3-32.1	.....

\* Only one case was contracted after 48 hours of age.

duration of individual cases. The size and distribution of the doses is shown further in table 6. In 89 per cent of the cases treatment was necessary for a maximum period of only 12 hours, during which not more than three doses were given. In the early part of this investigation, it was observed that when the initial dose was large and subsequent doses were smaller the rate of recovery seemed to be more rapid than when the same total quantity was given in equal doses; hence the adoption of a schedule in which the dosage size was reduced as treatment progressed. The over-all average amount of sulfaguanidine given per 100 pounds body weight in the first three doses was 7.0 grams, 5.0 grams and 4.0 grams, and the average time intervals between doses was five hours between the first two and six hours between the last two.

Presumably the rapid course of the scours necessitates the establishment of a high concentration of sulfaguanidine in the gastro-intestinal tract at an early stage to prevent irreparable damage to the tissues. Thus a large initial dose is deemed advisable. Furthermore, the scouring calf sometimes con-

TABLE 6

*Quantity and distribution of therapeutic doses of sulfaguanidine given for scours in new-born calves*

Maximum No. of doses given	Dosage/cwt. of calf		Cases treated	Ave. time between doses
	Average	Range		
	<i>grams</i>	<i>grams</i>	<i>per cent</i>	<i>hours</i>
1	6.8	3.3- 9.8	39	.....
2	6.3	2.8-10.2*	25	5
3	5.3	2.0-10.0	25	6
4	5.3	1.8- 9.8	5	8-12†
5	4.5	2.5-10.0	4	6
6	5.3	3.4- 8.5	2	6

\* One severe case, not included, received a 19-gram initial dose from which the response was favorable.

† Discontinued P.M. and resumed following A.M. in most cases.

tinues to weaken after the first dosing, and the difficulty of swallowing a subsequent dose frequently increases. The weaker the calf becomes the more relaxed the esophageal groove is likely to be, permitting the passage of either a portion or all of the sulfaguanidine into the rumenoreticular cavity, where the chemical is ineffective.

Even though prophylactics did not prevent scours in all cases, they always reduced the severity of the malady. The relationship between the prophylactic measures and the responses from therapeutic dosage with sulfaguanidine is indicated in table 7. Although serum *per se* was ineffective as a preventive, it was a beneficial adjuvant in sulfaguanidine therapy. The high efficiency of sulfonamide prophylaxis reduced the incidence of scours to the extent that only a small number of cases needed treatment.

TABLE 7

*The relation of prophylactic treatment to effectiveness of sulfaguanidine therapy in scours of new-born calves*

Prophylaxis	Therapeutically treated with sulfaguanidine	
	No. of calves	Mortality rate
None	35	<i>per cent</i> 20.0
Serum	18	5.6
Sulfonamides	3	0.0

## DISCUSSION

The recurring cycle of scours among new-born calves consists of eruptive outbreaks of high incidence for a short period followed by sporadic cases during a longer period. This suggests a marked variation in the susceptibility of the calves and/or in the virulence of the causal agents. The specific factors involved in this variability have not been disclosed, but in general practice scrupulous sanitation of the surroundings and proper nutrition of the calf are indispensable measures in breaking the cycle. These basic procedures used in conjunction with sulfaguanidine treatments have proved highly effective in reducing the incidence and the severity of the malady.

The efficacy of sulfaguanidine prophylaxis and therapy depends on numerous interrelated factors among which the more important ones probably are prenatal nutrition, congenital abnormalities, portal of entry of causative elements, time of infection (ante- or post-natal), virulence and quantity of infective agents resident in the herd environment, age when calf first nurses, quality and amount of colostrum consumed and regularity of consumption. Of these, the early post-natal nutrition merits special emphasis.

Since Smith and Little (24) focused attention on the significance of colostrum in combatting scours of new-born calves, other investigators (20,

22, 27) have associated the vitamin A activity of this secretion with its protective characteristics. Therefore, Stewart and McCallum (26) reasoned that the occurrence of scours and allied infections might be considerably lessened in herds if the vitamin A activity of the colostrum of all the cows could be maintained at a high level so that each calf would receive a sufficiency of this vitamin at birth. It was further assumed that increasing the vitamin A activity of the gestation diet, by feeding either carrots or cod-liver oil, would increase the level of this vitamin in the colostrum. Results from investigations based on these premises were negative. These findings in conjunction with general observations made in the study herein reported indicate that in a well-fed herd, the incidence of scours among the new-born calves is not related to the gestation diets of the dams.

It has been noted further that whenever the udders of individual dams fail to develop and distend normally, the immediate post-partum mammary secretions usually do not possess the properties characteristic of colostrum, and the new-born offspring have a low degree of resistance to scours. In these cases, it is advisable to increase the prophylactic doses of sulfaguandine and to feed colostrum from another cow. These practical observations indicate the need for additional fundamental information on the relation of the potency of the immunizing agents of colostrum to its chemical composition and physical properties.

Frequent consumption of small amounts of colostrum tends to prevent abomasal over-distension, which predisposes the calf to scours (25, 32). Either delay in the initial nursing or increasing the intervals between periods of consumption stimulates overfeeding and distension if the quantity of food is unlimited. If the calf is permitted to nurse at will during the first three to five days, which in many herds is the time it normally remains with its dam, scours resulting from overconsumption of colostrum is uncommon.

In the treatment of diarrhea of new-born calves failures of sulfaguandine therapy may be ascribed to several factors among which delay in treatment, early age at which scours is contracted, presence of excessive amounts of colostrum in the digestive tract and chemo-toxicity are probably the most frequently involved. The absence of pronounced premonitory symptoms and the rapid course of the disease occasionally result in the malady reaching an advanced stage before treatment is started. Cases of this type are encountered most often in the morning, having gone unobserved through the night. This hazard further emphasizes the importance of preventive measures in herds where the disease is entrenched.

The earlier the diarrhea develops after birth the more difficult it is to overcome. Fortunately, the manifestation of scours in calves less than 24 hours of age is exceptional. These early cases may be ascribed either to a possible *in utero* infection or to an inhibition of the normal protective

activity within the calf (25). The latter may be brought about by inherent morphological and functional defects of the digestive tract and/or by delayed feeding of colostrum. Furthermore, in treating these cases of diarrhea the sulfaguanidine may be absorbed so rapidly that it becomes difficult to establish a bacteriostatic or bactericidal concentration in the alimentary canal. This possibility merits further exploration.

Another aspect of the problem of treating the calf less than 24 hours of age and the calf that is in an advanced stage of diarrhea is that of toxicity from the sulfaguanidine (29). The increased permeability of the intestines in these cases may permit the passage of the chemical in sufficient amounts to develop toxic concentrations in the system. Dehydration accompanying diarrhea possibly further augments the toxic reactions, thus increasing the complications and fatalities (1). This potential danger should be considered when sulfaguanidine is used in treating diarrhea of calves.

The therapeutic efficiency of sulfaguanidine is exceptionally low in scouring calves that are bloated. This symptom usually indicates digestive failures as evidenced by the retention of large amounts of curd in the abomasum. In these cases portions of the doses are diverted into the rumenoreticular cavity, thus lowering the efficiency of the treatment. In addition it is possible that the coagulated material in the gastric secretions obstructs the passage of the medicine into the intestines and/or otherwise reduces its effectiveness. The *a priori* relationships of the factors involved in scours, bloat and indigestion are obscure, but the deleterious effects of loading the digestive tract of the scouring calf with milk are obvious. Therefore, during the acute stages of the diarrhea of new-born calves, it is essential that all food be withheld.

Although the therapeutic efficiency of sulfaguanidine as indicated in the experimental results is relatively high, its prophylactic use should be given precedence in herds where scours of the new-born calves prevails. Whenever therapeutic measures are necessary, it is well to give the sulfaguanidine as a drench. Giving liberal amounts of warm water between doses may retard the rate of dehydration and lessen the concentration of the chemical in the urine. Though no experimental observations were made, it is probable that parenteral administrations of isotonic solutions of either glucose or sodium chloride may be valuable supportive treatments to guard against excessive dehydration. During the convalescent period, meticulous care sometimes is necessary to avert complications, particularly pneumonia. Anorexia and general weakness occasionally necessitate force-feeding, in which case supplementing the colostrum milk with cod liver oil seems to accelerate recovery.

#### SUMMARY

1. An acute infectious and contagious diarrhea ("white scours") of new-born dairy calves was investigated clinically.

2. Scrupulous sanitation of the surroundings and adequate nutrition of the calf during the immediate post-natal period are regarded as fundamental in the control of scours of new-born calves but such measures alone are not adequate to control the disease under general herd conditions.

3. No recognizable reduction in the incidence of scours resulted from either giving cod liver oil as a dietary supplement or injecting a commercial anti-scour serum as a prophylactic measure.

4. Sulfaguanidine given in proper doses was effective prophylactically and therapeutically. A rule-of-the-thumb procedure could not be followed in all dosing.

5. As a prophylactic agent, it is recommended that sulfaguanidine in doses of approximately two grams be given to the calf at the following intervals: first dose, shortly after first colostrum consumption, which should not be later than two hours after birth; second dose, six to eight hours later; subsequent doses, morning and night through the second or third day. Doses may be reduced to one gram after the first 36 hours.

6. The following amounts of sulfaguanidine, per 100 pounds body weight of calf, given at four to six hour intervals are recommended as a guide for treatment: first dose, 7 grams; second dose, 5 grams; third and subsequent doses, 4 grams. These doses should be varied according to the condition and response of the calf. During the first 24 hours after the first clinical symptoms of scours are manifested, all feed should be withheld from the calf and liberal amounts of warm water should be given. Discontinue the sulfaguanidine therapy as soon as scours subsides.

7. In view of the dangers from complications associated with diarrhea of the new-born calf, prophylactic measures should be given precedence over therapeutic control except in herds where only sporadic cases occur.

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## RELATION OF VARIOUS FACTORS TO THE BREEDING EFFICIENCY OF DAIRY ANIMALS AND TO THE SEX RATIO OF THE OFFSPRING

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Breeding efficiency is an important economic factor in maintaining or developing a dairy herd, especially when proved sires are used, and also in regulating the production of the herd to meet market conditions. Although a proved sire is usually expensive to purchase, his ultimate cost is determined largely by the length of time he maintains his breeding efficiency.

The breeding records of the dairy herd maintained by the Bureau of Dairy Industry at Beltsville, Md., have been studied in an effort to determine the relationship of various factors to the breeding efficiency of sires and dams and also to the sex ratio of the resulting offspring.

The Beltsville herd, which was established in 1912, consists largely of purebred Holstein-Friesian and Jersey cattle, a small group of Red Danish cattle that was added in 1936, and a few grade animals. The data used in this study consist largely of the records of the herd from 1919 to 1942, inclusive.

### MANAGEMENT OF THE HERD

Since 1918 the major portion of the herd has been devoted to long-time breeding projects (1), which call for the use of a succession of unrelated proved sires. Linebreeding and inbreeding experiments are also being conducted.

An effort has been made to keep the management of this herd as uniform as possible. All cows are put on official test (365 days) during their first lactation and again after they are 5 years old. Test cows are kept in box stalls, milked three times daily, and are not given access to pasture. When the cows are not on test, they are milked twice daily in stanchions and are provided with pasture when available. All feeding is done according to feeding standards, the ration consisting chiefly of corn silage, alfalfa hay, and a grain mixture containing 15.5 per cent of digestible protein. During the test year the cows are bred 5 to 6 months after they calve, but during other lactations they are bred 3 to 4 months after calving.

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Careful attention is given to the care of the bulls. They are adequately housed and fed and are given 2 hours of exercise daily. Sprouted oats is a part of the bulls' ration.

Various diseases have occurred in the herd during the period covered by this study, including outbreaks of both tuberculosis and brucellosis, and at one time some evidence of trichomoniasis.

#### RESULTS OF THE STUDY

*Effect of age of sire on breeding efficiency.* In studying the effect of age on breeding efficiency, the males and females were first considered separately.

TABLE 1  
*Effect of age of sire on breeding efficiency*

Age of sire (years)	Total services	Services to non-breeders	Services to fertile cows	Total conceptions	Services per conception
Under 1	30	2	28	10	2.80
1	996	245	751	336	2.24
2	918	210	708	337	2.10
3	748	174	574	254	2.26
4	651	141	510	199	2.56
5	749	163	586	220	2.66
6	989	254	735	272	2.70
7	1,348	289	1,059	416	2.55
8	1,446	291	1,155	385	3.00
9	1,325	285	1,040	320	3.25
10	1,078	310	768	242	3.17
11	584	95	489	155	3.15
12	370	116	254	90	2.82
13	148	61	87	31	2.81
14	145	72	73	29	2.52
15	107	43	64	26	2.46
16	56	21	35	16	2.19
17	5	2	3	1	3.00
Total or average	11,693	2,774	8,919	3,339	2.67

Table 1 shows the effect of the age of a sire on his breeding efficiency, as calculated from services to fertile cows only.

In general the number of services per conception shows a slight upward trend between the ages of 1 and 12 years. The number of cows served by bulls under 1 year of age and at 12 years and older is so small that uniformity cannot be expected. One break in the curve occurs at the age of 7 years. Most of the proved sires that were purchased came into the herd at about 7 or 8 years of age. It was thought that possibly the change in environment of these purchased sires had a temporary beneficial effect upon their efficiency. To test this theory, a tabulation was made of the breeding records of eight Beltsville-bred sires that were in regular service at the station during their entire lifetime (in all cases at least 9 years) (see table 2). These eight sires show a greater increase in breeding efficiency from

the age of 6 to 7 years than is shown by the sires in table 1. We have no explanation for the increase in breeding efficiency from 6 to 7 years of age by either group of sires.

*Effect of age of female on breeding efficiency.* Table 3 shows the effect of age of the female on breeding efficiency. The data are based on the age of the cows at the time of conception. All breedings to fertile sires are included. In cases where the records show that a bull was obviously declining in fertility toward the end of his active service he is considered sterile after the date of his last successful service, and breedings after that date are not charged against the cows.

Here again the older groups are small and the figures cannot be considered conclusive. However, until females are 9 years of age, their age has

TABLE 2  
*Average breeding efficiency of 8 Beltsville-bred sires at various ages*

Age of bulls (years)	Total ser- vices	Services to non- breeders	Services to fer- tile cows	Total concep- tions	Services per-con- ception
1	175	63	112	48	2.33
2	244	72	172	86	2.00
3	299	81	218	86	2.53
4	249	46	203	76	2.67
5	299	48	251	83	3.02
6	440	119	321	100	3.21
7	368	100	268	105	2.55
8	219	65	154	61	2.52
9	102	37	65	13	5.00
10	64	35	29	13	2.23
Total or average	2,459	666	1,793	671	2.67

little apparent effect upon their breeding efficiency. These figures do not show the marked decrease in number of services required for conception after 2 years of age that was found by Morgan and Davis (3). The 2-3-year-old group is made up largely of cows being bred for their second gestation, but a considerable number of heifers being bred for their first gestation are also included in this age-group. Most of the heifers in this herd are bred first at about 15 months of age, so it is obvious that these "carry-overs" required a large number of services. Probably this factor is a disturbing influence among the younger age-groups. The 1-2-year-old group required an average of 2.55 services per conception, while the 2-3-year-old group required 2.57 services per conception. If the first-gestation heifers are taken out of the 2-3-year-old group and included in the 1-2-year-old group, the figures become 2.88 services per conception for the first-gestation group and 1.97 for the 2-3-year-old cows. Possibly this affords a truer measure of the breeding efficiency of younger cows than a grouping strictly according to ages.

TABLE 3  
*Effect of age of female on breeding efficiency*

Age of cows (years)	Total services	Number cows conceiving on service No.										Total conceptions	Services per concep.
		1	2	3	4	5	6	7	8	9	10	11 and over	
1-2	2,286	398	165	120	74	47	39	30	9	6	3	6	2.55
2-3	1,573	293	119	65	41	34	15	8	9	5	11	12	2.57
3-4	1,255	247	100	57	30	29	15	10	10	2	5	4	2.47
4-5	1,233	180	98	63	38	20	17	6	6	7	5	9	2.75
5-6	1,023	153	73	45	18	18	11	7	6	3	2	14	2.92
6-7	643	134	48	32	15	15	11	5	1	4	.....	3	2.40
7-8	552	78	31	35	18	7	4	4	3	3	2	5	2.91
8-9	327	48	26	15	7	8	2	4	3	1	.....	3	2.79
9-10	250	36	14	7	5	10	6	2	3	.....	1	1	2.94
10-11	106	14	10	9	3	1	.....	2	.....	.....	1	1	2.65
11-12	67	8	4	4	4	.....	1	.....	1	1	.....	.....	2.91
12-13	27	1	2	2	1	.....	2	.....	.....	.....	.....	.....	3.38
13-14	10	2	.....	1	.....	1	.....	.....	.....	.....	.....	.....	2.50
14-15	7	4	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	1.40
Total or average	9,359	1,596	690	456	254	190	123	78	51	32	29	58	2.63
Per cent of total*		44.9	19.4	12.8	7.1	5.3	3.5	2.2	1.4	0.9	0.8	1.6	

\* 77.1% conceived on first 3 services; 89.6% conceived on first 5 services.

Table 3 also shows the number of conceptions that occurred from various numbers of services for each age-group. It will be noted that, for all ages, 77.1 per cent conceived in three services or less and 89.6 per cent conceived in five services or less. These figures may throw some light on the question of how long a cow should be retained in an effort to get her with calf. Of course, many factors must be considered, but unless the cow is particularly valuable it seems doubtful that more than five services would be warranted in a commercial herd. Normally the time required for five services is approximately 3 months, and some additional time must elapse before pregnancy can be definitely determined. These percentages are particularly impressive in view of the fact that in this herd the cows that fail to conceive

TABLE 4  
*Effect of age of both parents on breeding efficiency*

Cows' gestation number	Bulls under 5 years			Bulls 5 years and over			All bulls		
	Number services	Number conceptions	Services per conception	Number services	Number conceptions	Services per conception	Number services	Number conceptions	Services per conception
1	802	421	1.90	1,309	431	3.04	2,111	852	2.48
2	385	206	1.87	1,122	497	2.26	1,507	703	2.14
3	271	137	1.98	910	397	2.29	1,181	534	2.21
4	183	101	1.81	726	295	2.46	909	396	2.30
5	139	66	2.10	508	230	2.21	647	296	2.19
6	82	40	2.05	344	152	2.26	426	192	2.22
7	53	23	2.30	210	85	2.47	263	108	2.44
8	23	15	1.53	84	40	2.10	107	55	1.95
9	7	5	1.40	65	26	2.50	72	31	2.32
10	.....	.....	.....	23	12	1.92	23	12	1.92
11	.....	.....	.....	10	5	2.00	10	5	2.00
12	.....	.....	.....	7	3	2.33	7	3	2.33
13	.....	.....	.....	1	1	1.00	1	1	1.00
14	.....	.....	.....	1	1	1.00	1	1	1.00
Total or average	1,945	1,014	1.92	5,320	2,175	2.45	7,265	3,189	2.28

promptly receive veterinary treatment. Such treatment would not be available in the average commercial herd.

*Effect of age of both parents on breeding efficiency.* A comparison of the breeding efficiency of young and old sires when bred to cows of varying ages is shown in table 4. The cows are grouped according to gestation rather than age. The data are omitted for all cows bred to more than one sire for a single conception. These figures show that the young bulls are more efficient than the older ones on cows of all ages, but their greatest margin over the older bulls was on the first-gestation heifers.

As in most of the other tabulations, the groups of older cows are small and the figures are therefore not conclusive. It cannot be assumed that all old bulls will not breed heifers efficiently. To illustrate this point a calculation was made of the breeding records of 4 Jersey bulls that were returned

TABLE 5  
*Effect of age of parents on sex ratio*

Age of sire (years)	Percentage of male calves born when cow's age at time of conception was											Total male prog- eny	Total female prog- eny	Per cent male prog- eny
	1 year	2 years	3 years	4 years	5 years	6 years	7 years	8 years	9 years	10 years	11 years and over			
1	48.7	55.2	67.9	44.8	43.5	38.5	50.0	66.7	66.7	100.0	.....	169	160	51.4
2	44.6	69.2	47.4	58.8	66.7	50.0	43.8	54.5	60.0	50.0	0.0	188	175	51.8
3	48.6	52.4	68.6	43.8	59.1	62.5	63.6	80.0	0.0	66.7	100.0	147	127	53.6
4	55.4	59.5	75.0	67.9	42.1	53.3	42.9	16.7	40.0	66.7	33.3	123	93	56.9
5	65.4	52.8	44.8	54.8	54.2	38.1	63.6	77.8	40.0	40.0	66.7	124	102	54.9
6	46.8	43.9	65.1	45.5	54.8	68.0	61.5	63.6	55.6	50.0	80.0	162	142	53.3
7	52.8	60.6	48.3	53.8	45.7	41.7	47.1	60.0	50.0	33.3	20.0	208	196	51.5
8	59.3	54.7	60.4	50.0	51.1	54.1	59.1	44.4	50.0	25.0	100.0	214	173	55.3
9	59.3	33.3	57.6	44.2	53.7	51.6	36.0	70.0	57.1	.....	50.0	168	169	56.3
10	54.3	57.9	62.8	50.0	52.0	44.0	75.0	72.7	50.0	50.0	.....	139	108	57.6
11	63.3	53.1	70.4	51.9	52.2	83.3	28.8	50.0	33.3	.....	.....	102	75	44.3
12	33.3	50.0	61.5	30.8	83.3	20.0	37.5	25.0	75.0	0.0	66.7	38	44	46.3
13 & over	0.0	63.2	50.0	37.5	50.0	44.4	27.3	16.7	71.4	25.0	60.0	45	51	46.9
Total or average	52.1	54.0	58.9	50.1	52.4	51.0	49.2	56.8	52.6	42.9	60.0	1,827	1,615	53.1

NOTE: Monosexual twins counted as one calf. Bisexual twins omitted.



to Beltsville after several years of service in cooperators' herds. Their average age at the time of starting service at Beltsville was 7 years 8 months. Their average breeding efficiency on 69 virgin heifers was 1.84 services per conception, or slightly better than the average for all bulls under 5 years of age.

In 1935 a practice was started of reserving certain young bulls for use with virgin heifers only. This was based on the theory that the herd bulls might be transferring infection from the older cows to the heifers, thus making it difficult to get them with calf. The average age of the young bulls was 3 years 1 month at the time of their last service to the virgin heifers. Seven of these bulls obtained 164 conceptions from 279 services, an average of 1.70 services per conception. This average is significantly lower than the average for all bulls under 5 years of age when bred to heifers.

*Effect of age of parents on sex ratio.* Table 5 shows the effect of the age of the parents on the sex ratio of the offspring. The tabulation is based on the age of each parent at the time of conception. There seems to be no indication that the age of the parents has any particular effect on the sex ratio of the offspring. The only conclusion from these figures is that the normal expectancy is a slight preponderance of males. This conforms to the findings of other workers.

*Effect of calving interval on breeding efficiency.* A theory has been advanced that a cow bred to calve 12 months after freshening will conceive more readily than one bred for a longer calving interval. Since it was the practice in this herd to breed the cows for a 15-month calving interval during test years and for a 12-month interval during other lactations, it is possible to compare these two calving intervals in the same herd. Among the Jerseys and Holsteins 432 cows required 2.54 services per conception when bred to calve in 15 months, while 431 cows required 2.69 services per conception when bred to calve in 12 months. This difference could hardly be called significant.

*Effect of season on breeding efficiency and sex ratio.* In order to determine whether the season of the year has any effect on breeding efficiency, the service record of each sire was compiled by months. The summary for all sires is given in table 6. The data show an increase in services per conception during July, August, and September. The number also increased during February and March. The seasonal trend in breeding efficiency at the Beltsville station is similar to that reported for the University of Nebraska herd (3), except that the periods of lowest efficiency occur about a month later at the Nebraska station than at Beltsville. (See figure 1.)

The period of lowest fertility during midsummer may be due in part to high temperatures and their direct physiological effect on the animals. Phillips *et al.* (4) in a study of seasonal variation in bull semen, report that most measures of quality in semen indicate it is of lower quality during the summer months than during the rest of the year. No explanation is avail-

able for the increase in services required for conception during the late winter season.

There is no evidence in the data to show that season has any effect on sex ratio.

*Yearly variation in breeding efficiency.* Table 7 shows the variation in breeding efficiency from year to year. This tabulation is based on the same figures as those in table 6, combined by years instead of by months. There appears to be a tendency for the fertility to be lowest in the years following those in which the greatest percentage of abortions occurred. This bears out the findings of Miller, Graves, and Fohrman (2) who found that fertility decreases after abortions caused by brucellosis, but not after abortions that result from other causes. In 1925 and 1926, when the percentage of abor-

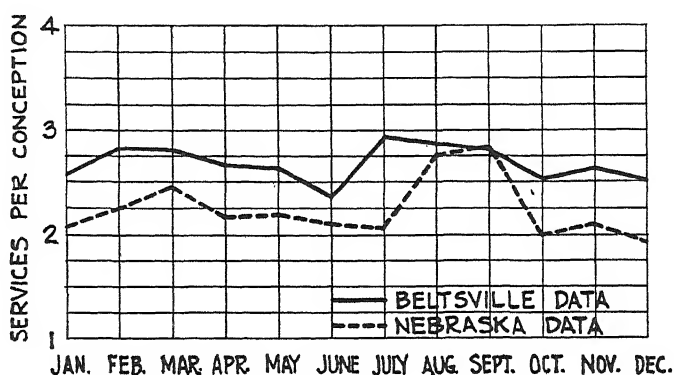


FIG. 1. Effect of season on breeding efficiency.

tions was very high in the Beltsville herd, brucellosis was responsible for a large proportion of the abortions.

#### BREEDING PROBABILITIES

Table 8 is a compilation of the breeding records of 725 cows in the Beltsville herd for which the entire life history is available. The breeding records of these cows are an indication of the number of replacements that must be made to maintain a dairy herd. For example, only 449 of the original 725 cows, or 61.9 per cent, were bred for the third calf, and only 393 of these conceived for the third time. There have been two serious outbreaks of disease in this herd in the past 25 years, and for this reason the figures may not be representative of an average cow population. Two factors, however, at least partially offset the effect of these disease epidemics: 1) No culling for low production was practiced, and 2) some of the cows that reacted to the tuberculosis and brucellosis tests were not disposed of immediately, but were isolated from the rest of the herd and retained for breeding purposes.

In the various tables presented in this study, the average number of services per conception is not always the same since the groups are composed

TABLE 6  
Effect of season of the year on breeding efficiency and sex ratio

Month of conception	Number cows bred	Total services	Services to fertile cows	Per cent of conceptions on service No.*				Total conceptions	Result of conception †			Services per conception
				1	2	3	4 and over		Living calves	Per cent abortions	Per cent male progeny	
Jan. ....	830	955	739	46.7	21.3	10.8	21.2	287	231	9.4	53.9	2.57
Feb. ....	794	899	695	47.3	21.1	13.4	18.2	247	192	14.2	50.9	2.81
Mar. ....	875	1,028	795	48.9	19.1	14.4	17.6	284	226	8.5	50.9	2.80
Apr. ....	923	1,061	825	56.6	15.8	10.6	17.0	311	252	9.6	54.2	2.65
May ....	950	1,097	864	48.0	19.9	11.9	20.2	327	260	10.7	53.0	2.64
June ....	851	954	725	48.2	17.3	10.1	24.4	307	255	6.2	51.9	2.36
July ....	796	918	703	50.6	18.0	10.9	20.5	239	196	7.5	52.0	2.94
Aug. ....	892	1,049	778	44.1	21.1	17.0	17.8	270	207	9.6	54.5	2.88
Sept. ....	884	1,017	761	46.7	23.0	13.3	17.0	270	214	8.5	53.8	2.82
Oct. ....	938	1,077	813	47.2	19.3	12.4	21.1	322	242	12.4	52.5	2.52
Nov. ....	842	947	732	52.0	21.3	13.4	13.3	277	224	7.2	58.8	2.64
Dec. ....	865	986	751	54.4	19.5	11.4	14.7	298	239	8.1	51.6	2.52
Total or average .....	.....	11,988	9,181	49.3	19.6	12.4	18.7	3,439†	2,738	9.3	53.2	2.67

\* Services to one sire only.

† Monosexual twins counted as one calf. Bisexual twins omitted.

+ 81.3% of conceptions obtained on first 3 services; 93.1% of conceptions obtained on first 5 services.

TABLE 7  
Yearly variation in breeding efficiency and sex ratio

Year	Number cows bred	Total services	Services to fertile cows	Per cent of conceptions on service No.*				Total conceptions	Result of conception†			Services per conception
				1	2	3	4 and over		Living calves	Per cent abortions	Per cent male progeny	
1917	1	1	1	100.0	.....	.....	.....	1	1	0.0	0.0	1.00
1918	12	25	25	25.0	50.0	25.0	.....	4	2	50.0	0.0	6.25
1919	35	48	38	86.4	4.5	9.1	.....	22	14	13.6	50.0	1.73
1920	44	83	48	77.3	9.1	9.1	4.5	22	18	13.6	52.4	2.18
1921	86	216	169	47.0	12.1	16.7	24.2	66	45	25.8	56.5	2.56
1922	98	268	215	51.7	18.3	10.0	20.0	60	48	15.0	43.6	3.58
1923	148	368	300	52.2	12.0	14.1	21.7	92	78	8.7	48.8	3.26
1924	134	323	224	58.4	11.2	7.9	22.5	89	70	16.9	51.2	2.52
1925	162	422	286	50.0	22.2	15.7	12.1	108	66	29.6	54.5	2.65
1926	179	470	360	46.0	17.7	14.5	21.8	124	81	25.8	53.1	2.90
1927	162	434	344	43.4	15.0	13.3	28.3	113	87	8.8	52.8	3.04
1928	199	403	330	60.2	13.5	8.3	18.0	133	104	10.5	63.5	2.48
1929	208	471	365	49.0	18.2	15.4	17.4	143	113	14.7	45.7	2.55
1930	266	639	474	48.2	22.3	12.0	17.5	166	132	9.4	54.7	2.86
1931	271	665	473	44.4	20.6	13.1	21.9	160	109	16.9	50.0	2.96
1932	289	783	541	49.4	22.2	12.2	16.2	180	136	10.0	52.1	3.01
1933	260	727	534	44.5	20.9	13.2	21.4	182	150	3.3	61.3	2.93
1934	256	644	515	47.7	14.0	10.4	27.9	193	159	7.3	55.5	2.67
1935	274	696	555	35.3	22.9	16.5	25.3	170	127	5.9	61.0	3.26
1936	215	480	373	47.3	25.7	9.0	18.0	167	131	8.4	52.6	2.23
1937	202	466	386	51.9	15.6	13.0	19.5	154	131	3.9	52.1	2.51
1938	243	598	441	49.7	24.5	9.7	16.1	155	139	1.9	51.0	2.85
1939	288	636	482	55.0	23.3	11.6	10.1	189	170	3.7	43.5	2.55
1940	346	838	672	47.2	19.6	14.8	18.4	271	236	5.9	52.1	2.48
1941	331	663	515	50.8	25.6	10.2	13.4	246	198	4.5	54.4	2.09
1942	229	621	515	52.0	19.2	13.1	15.7	229	193	3.5	55.0	2.25
Total or average	.....	11,988	9,181	49.3	19.6	12.4	18.7	3,439	2,738	9.3	53.2	2.67

\* Services to one sire only.

† Mono sexual twins counted as one calf. Bisexual twins omitted.

TABLE 8  
*Life-time breeding records of 725 cows in the Beltsville herd*

Gestation number	Number cows not bred for this gestation	Cows bred		Number of non-breeders	Number cows failed to conceive*	Number cows conceived	Per cent of cows conceiving on service No.				Services per conception
		Number	Per cent				1	2	3	4	
1	....	725	100.0	27	21	677	33.4	14.8	12.6	32.7	8.23
2	83	594	81.9	41	25	528	41.4	17.5	12.5	17.5	2.41
3	79	449	61.9	39	17	393	39.2	17.8	8.5	22.0	2.83
4	80	313	43.2	21	11	281	37.1	20.8	13.1	18.8	2.67
5	53	228	31.4	18	11	199	40.8	18.4	11.4	16.7	2.35
6	41	158	21.8	19	12	127	35.4	11.4	19.0	14.6	2.50
7	32	95	13.1	15	5	75	32.6	14.7	10.5	21.0	2.73
8	18	57	7.9	8	5	44	35.1	12.3	15.8	14.0	2.30
9	13	31	4.3	6	1	24	19.4	22.6	19.4	16.2	2.67
10	9	15	2.1	4	2	9	26.7	13.3	6.7	13.4	2.33
11	4	5	0.7	1	.....	4	40.0	20.0	.....	20.0	2.25
12	1	3	0.4	.....	1	2	.....	.....	66.7	.....	3.00
13	1	1	0.1	.....	.....	1	100.0	.....	.....	.....	1.00
14	.....	1	0.1	.....	.....	1	100.0	.....	.....	.....	1.00
Total or average	.....	2,675	.....	199	111	2,365	37.2	16.7	12.3	22.2	2.76

\* This column includes cows that received one or more services but died or were eliminated from the herd for causes other than sterility.

of different animals. As was mentioned before, the early records of this station were not always complete, which rendered some of the data suitable for some types of calculation but not for others.

#### SUMMARY

The breeding records of the Bureau of Dairy Industry herd at Beltsville, Md., have been studied from the standpoint of breeding efficiency and sex ratio of offspring as influenced by the ages of the cows and bulls and by the season of the year.

The sires showed a gradual lessening of breeding efficiency with advancing age, with the exception of the 7-year-old group. No explanation can be offered for the decrease that occurred in number of services per conception at this age.

After the first gestation, age had little apparent effect upon the breeding efficiency of the cows. Heifers being bred for the first time required more services than the older cows.

Bulls over 5 years of age showed a distinctly higher number of services per conception than did young bulls when bred to heifers being bred for their first gestation. Young bulls that were bred exclusively to virgin heifers proved even more efficient than the whole group of young bulls.

Breeding efficiency of the cows was not appreciably affected by length of calving interval.

The most noticeable effect of season on breeding efficiency was the relatively large number of services required for conception during midsummer, followed by a sharp decrease in the fall.

Data showing the yearly variation in breeding efficiency indicate that there was a tendency for the years of lowest breeding efficiency to follow years when the percentage of abortions was highest.

A tabulation of the complete breeding life histories of 725 cows in the Beltsville herd indicates the probable breeding losses in a dairy herd from various causes.

There is no evidence that any of the factors studied influenced the sex ratio of the offspring.

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# BACTERIOLOGICAL EVALUATION OF ICE CREAM<sup>1</sup>

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The standard plate count, usually with incubation at 37° C., has been used almost to the exclusion of other possible means for determination of "total" numbers of bacteria in ice cream. The studies reported here seek to establish whether certain of the other methods used for dairy products may be substituted.

## I. THE RESAZURIN AND METHYLENE-BLUE REDUCTION TESTS

Although both the resazurin and the methylene-blue reduction tests have been used quite widely on unpasteurized milk and to some extent on pasteurized milk and cream, they apparently have not been employed extensively for bacteriological evaluation of ice cream or ice cream mix. The simplicity and inexpensiveness of these tests commend them to use where applicable.

### METHODS

Factory-packed pint samples of ice cream, each from a different manufacturer, were shipped with dry-ice refrigeration to the laboratory, where they were held in the ice cream hardening room until analyzed. Data in parts II and III were obtained from the same set of samples. The ages of the different samples were not known. The plate counts were obtained by using the volumetric procedure (1). The methylene-blue tests were made according to the Standard Methods procedure for milk, using methylene-blue thiocyanate tablets (1). The tubes were examined at one-half hour intervals, but they were not inverted at examination. The resazurin tests were made by adding 0.1 ml. of a 0.05 per cent solution of Eastman resazurin to each 10 ml. quantity of melted ice cream, which then was handled in the same manner as the methylene-blue tests. The time required for the milk to become a definite pink, free from blue, and the color changes at one and three hours were recorded.

Thornton and Hastings (9) reported the presence of cysteine hydrochloride accelerated dye reduction. In one series of the methylene blue tests, 0.2 ml. of freshly prepared N/10 cysteine hydrochloride was added to the ice cream-dye mixture before incubation. This amount was found by

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TABLE 1  
*Relationship of standard plate count and resazurin reduction time*

Standard plate count per ml.	No. of samples with resazurin reduction time (min. to pink stage) of										Totals
	0-60	90-120	150-180	210-240	270-300	330-360	390-420	450-480	510-540	More than 540	
10,000 or less	1	.....	.....	.....	3	5	2	23	4	9	47
10,100-25,000	.....	.....	.....	1	5	12	5	12	1	1	37
26,000-50,000	.....	1*	.....	3	5	3	.....	3	.....	1	16
51,000-100,000	.....	1	2	1	6	4	3	4	2	.....	23
101,000-200,000	.....	.....	2	4	6	4	.....	1 <sup>a</sup>	1	.....	18
201,000-500,000	.....	.....	3	2	2	.....	.....	.....	.....	.....	7
510,000-1,000,000	2*	1	3	3	.....	.....	.....	.....	.....	.....	9
1,010,000-3,000,000	1	4	4	.....	.....	.....	.....	.....	.....	.....	9
3,100,000-10,000,000	4	4	.....	.....	.....	.....	.....	.....	.....	.....	8
Greater than 10,000,000	5	1	.....	.....	.....	.....	.....	.....	.....	.....	6
Totals	13	12	14	14	27	28	10	43	8	11	180

\* High direct microscopic count.



preliminary experiment to increase the rate of reduction without causing extremely rapid decolorization.

### RESULTS

A general relationship between standard plate count and resazurin reduction tests, using the time required for attainment of a pure pink color as the resazurin end-point, is apparent from the data presented in table 1. Had a standard of greater than 240 minutes to reduce the dye to the pink end-point been adopted, only two of 39 samples with plate counts about 200,000 per ml. would have been accepted; and ten samples with counts below 100,000 per ml. would have been rejected. One of the latter samples had a

TABLE 2  
*Relationship of resazurin color change after incubation at 37° C. for 1 and 3 hours and standard plate count on 177 samples of ice cream*

Standard plate counts per ml.	Number of samples after 1 hour incubation falling in color class				Totals	Number of samples after 3 hours incubation falling in color class			
	1*	2	3	4		1*	2	3	4
10,000 or less .....	42	2 <sup>b</sup>	1 <sup>c</sup>	1	46	38	6 <sup>b</sup>	1 <sup>c</sup>	1
10,100-25,000 .....	37	1	.....	.....	38	29	9	.....	.....
26,000-50,000 .....	13	4	.....	.....	17	9	7	.....	1 <sup>d</sup>
51,000-100,000 .....	19	3	.....	.....	22	12	7	3	.....
101,000-200,000 .....	13	4	.....	.....	17	5	8	3	1
201,000-500,000 .....	5	1	.....	.....	6	.....	3	2	1
510,000-1,000,000 .....	5	2	2	.....	9	.....	2	2	5
1,010,000-3,000,000 .....	1	7	1	.....	9	.....	.....	4	5
3,100,000-10,000,000 .....	.....	2	3	2	7	.....	.....	1	6
Greater than 10,000,000 .....	.....	1	1	4	6	.....	.....	.....	6
Totals .....	135	27	8	7	177	93	42	16	25

\* 1 = no color change.

2 = definite change to lavender.

3 = pink devoid of lavender.

4 = white.

<sup>b</sup> = two samples with high direct microscopic counts.

<sup>c</sup> = one sample with high direct microscopic count.

<sup>d</sup> = abnormal color change in dye.

very high direct microscopic count, indicating that the resazurin test may have been more accurate than the plate count in this instance. Direct microscopic counts were not available on most of the remaining samples which would have been rejected. No other end-point, with the possible exception of 180 minutes, would have given a comparable degree of separation of the high-count samples from those with low counts.

The data of table 2 show that color changes at one hour failed to permit separation of the samples into satisfactory groups based upon plate counts. The plate counts of the four classes established by color change at three hours overlapped considerably from class to class; but those samples in which the indicator had turned either definite pink or colorless in three

TABLE 3  
*Relationship of standard plate count and methylene-blue reduction time*

Standard plate count per ml.	No. of samples with methylene-blue reduction time (min.) of										Totals
	0-60	90-120	150-180	210-240	270-300	330-360	390-420	450-480	510-540	> 540	
10,000 or less	.....	1	.....	.....	.....	3	1	26	4	12	47
10,100-25,000	.....	.....	.....	.....	1	5	13	13	4	1	37
26,000-50,000	.....	1	.....	1	3	4	2	3	.....	2	16
51,000-100,000	.....	.....	.....	2	5	4	4	4	2	2	23
101,000-200,000	.....	.....	1	2	4	4	2	4	.....	1	18
201,000-500,000	.....	.....	1	2	2	1	1	.....	.....	.....	7
510,000-1,000,000	.....	1	2	2	4	.....	.....	.....	.....	.....	9
1,010,000-3,000,000	.....	3	1	4	1	.....	.....	.....	.....	.....	9
3,100,000-10,000,000	1	3	2	.....	1	1	.....	.....	.....	.....	8
Greater than 10,000,000	4	1	1	.....	.....	.....	.....	.....	.....	.....	6
Totals	5	10	8	13	21	22	23	50	10	18	180

TABLE 4  
*Relationship of standard plate count and methylene-blue reduction time with cysteine added*

Standard plate count per ml.	No. of samples with methylene-blue reduction time (min.) of										Totals
	30	60	90	120	150	180	210	240	270	300 or over	
10,000 or less	3	1	3	3	4	2	6	11	.....	5	38
10,100-25,000	.....	7	6	4	3	6	..	2	.....	3	31
26,000-50,000	2	2	3	3	2	3	1	1	.....	.....	17
51,000-100,000	.....	4	4	.....	4	4	1	1	1	2	21
101,000-200,000	.....	6	4	3	1	2	.....	.....	.....	.....	16
201,000-500,000	1	2	1	1	.....	.....	.....	.....	.....	.....	5
510,000-1,000,000	1	.....	4	1	.....	.....	.....	.....	.....	.....	6
1,010,000-3,000,000	2	1	2	1	.....	1	.....	.....	.....	.....	7
3,100,000-10,000,000	1	2	.....	1	.....	.....	.....	.....	1	.....	5
Greater than 10,000,000	4	.....	1	.....	.....	.....	.....	.....	.....	.....	5
Totals	14	25	28	17	14	18	8	15	2	10	151

hours were bacteriologically unsatisfactory by either the plate count or the direct microscopic count in nearly all instances. This was true especially of those samples which had reduced the dye to the colorless end-point.

A general relationship between methylene-blue reduction time and standard plate count on 181 samples of ice cream is shown in table 3, although the spread in counts in each dye reduction class is considerable. Segregation on the basis of methylene-blue reduction times of samples with either high or low plate counts does not appear possible without the misplacing of a considerable number of samples, particularly some with relatively high counts.

The data on 151 samples presented in table 4 indicate that a considerable decrease in methylene-blue reduction time resulted from the addition of cysteine hydrochloride; but the relationship between results of the usual methylene-blue reduction test and the standard plate count was decreased considerably by this addition. The results show little promise of practical application.

#### DISCUSSION

The possible limitations of the plate count as a basis of evaluation of other bacteriological tests are recognized, but other bases of evaluation available at present are subject to at least an equal number of potential limitations. The common use of the plate count for bacteriological control of ice cream makes desirable evaluation in terms of the plate count of newer methods such as reduction tests.

The data herein reported indicate that the resazurin reduction test, employing either the time required for the dye to become a definite pink or the color after incubation for three hours as the end-point, has possibilities as a means of segregating the majority of the ice cream samples with high standard plate counts from most of the samples with relatively low counts.

The methylene-blue reduction test appears to be less suited than the resazurin test as a simple method of determining the bacterial content of ice cream. Possibly the conditions of ice cream storage are such that the flora of the product is capable of only mild reducing activity, even when present in considerable numbers; and the more easily reduced resazurin is affected to a greater degree by the same population than is the less easily reduced methylene blue. Addition of cysteine hydrochloride to the methylene-blue reduction tests hastens reduction but weakens the relationship between numbers of bacteria and time of reduction.

#### CONCLUSIONS

1. The resazurin test, using either time for the color to become completely pink or the color of the indicator after three hours as the end-point, showed possibilities as a means of segregating samples of ice cream with high standard plate counts. The one-hour test showed little promise.

2. Segregation on the basis of the methylene-blue reduction test of ice cream samples with high plate counts did not seem practical upon the basis of results obtained in this study.

3. Addition of 0.2 ml. of N/10 cysteine hydrochloride to each methylene-blue test shortened the reduction time but disrupted the relationship between reduction time and standard plate count.

## II. PLATE COUNTS AT 45° AND 55° C.

Although plate counts employing incubation temperatures above 37° C. have been used with some success in the bacteriological evaluation of milk and cream, such procedures apparently have not been employed to any extent in testing ice cream. The studies reported here attempt evaluation of plate counts at 45° and 55° C. for determination of bacteriological quality of ice cream.

### METHODS

The volumetric procedure (1) was employed in preparation of all plates. Duplicate plates of each dilution of each sample were incubated for  $48 \pm 3$  hours at 37°, 45° and 55° C.

### RESULTS

The data showing the relationship between plate counts at 37° C. and 55° C. for 121 samples are presented in table 5. Of the 16 samples having counts at 55° C. in excess of 10,000 per ml., 10 had 37° C. counts of more than 300,000 per ml., while only 24 of the total of 121 samples had 37° C. counts in this range. Some tendency for high counts at 55° C. to be associated with high counts at 37° C. thus was indicated. The relatively low general level of thermophilic bacteria in ice cream may be associated with

TABLE 5

*Relationship of plate counts at 37° and 55° C. on 121 samples of ice cream*

Plate counts per ml. at 37° C.	No. of samples with 55° C. counts per ml. of						Totals
	Less than 1,000	1,000-3,000	3,100-10,000	10,100-30,000	31,000-100,000	101,000-300,000	
Less than 1,000 .....	3	.....	1	.....	.....	.....	4
1,000-3,000 .....	3	2	1	.....	.....	.....	6
3,100-10,000 .....	16	4	5	1	1	.....	27
10,100-30,000 .....	14	8	.....	.....	1	.....	23
31,000-100,000 .....	16	3	4	2	.....	.....	25
101,000-300,000 .....	10	.....	1	.....	1	.....	12
310,000-1,000,000 .....	4	2	.....	1	3	.....	10
1,010,000-3,000,000 .....	4	.....	.....	1	1	.....	6
3,100,000-10,000,000 .....	1	1	1	1	1	1	6
More than 10,000,000 .....	1	.....	.....	1	.....	.....	2
Totals .....	72	20	13	7	8	1	121

TABLE 6  
*Relationship of plate counts at 37° and 45° C. on 123 samples of ice cream*

Plate counts per ml. at 37° C.	No. of samples with counts at 45° C. of									Totals
	Less than 1,000	1,000- 3,000	3,100- 10,000	10,100- 30,000	31,000- 100,000	101,000- 300,000	310,000- 1,000,000	1,010,000- 3,000,000	3,100,000- 10,000,000	
Less than 1,000	4	2	1	1	1	1	1	1	1	4
1,000-3,000	2	13	11	6	1	1	1	1	1	6
3,100-10,000	4	7	6	10	5	3	1	1	1	28
10,100-30,000	1	2	8	3	5	4	1	2	1	23
31,000-100,000	1	1	1	2	1	1	1	1	1	25
101,000-300,000	1	1	1	1	1	2	1	1	1	13
310,000-1,000,000	1	1	1	1	1	2	1	1	1	10
1,010,000-3,000,000	1	1	1	1	2	1	1	1	1	6
3,100,000-10,000,000	1	1	1	1	2	1	1	1	1	6
More than 10,000,000	1	1	1	1	1	1	1	1	1	2
Totals	12	26	29	22	15	10	4	4	1	123

the tendency for organisms of this type to die out at low temperatures, as has been demonstrated for milk (3, 6, 8).

The relationship of plate counts at 37° C. to those at 45° C. for 123 samples is shown in table 6. The data indicate a pronounced tendency for high counts at one temperature to be associated with high counts at the other temperature, with comparatively few exceptions to the general relationship. The conclusion seems justified that counts at 45° C. would not furnish sufficient additional information over that obtained from counts at 37° C. to justify the routine use of both temperatures.

#### DISCUSSION

Plate counts at 45° C. and 55° C. apparently furnish little information not obtained by plate counts at 37° C. on ice cream. In view of the known effect of low-temperature refrigeration on the counts of thermophilic bacteria in pasteurized milk, the comparatively small numbers of these organisms in ice cream of the type studied here are not unexpected. Routine detection in ice cream of organisms able to grow at 45° and 55° C. does not seem to be justified upon the basis of data obtained in this study.

#### CONCLUSIONS

1. The samples of ice cream examined contained few thermophilic bacteria.
2. Plate counts at 45° and 55° C. furnish little information in addition to that obtained with incubation at 37° C.

#### III. THE DIRECT MICROSCOPIC COUNT

Although Fabian (4) and Fay (5) outlined procedures for the direct microscopic method as applied to ice cream, neither of these investigators presented data concerning the quantitative significance of his results. Direct microscopic procedures for ice cream are presented in the seventh and eighth editions of Standard Methods for the Examination of Dairy Products (1, 2), with the notation that basis for quantitative interpretation is not available. The studies reported here attempt evaluation of the direct microscopic procedure for determination of bacteriological quality of ice cream.

#### METHODS

Preparations for direct microscopic counts were made by smearing 0.01 ml. of melted sample measured by capillary pipette over an area of 1 cm.<sup>2</sup> The fixing and staining procedures outlined for ice cream in Standard Methods (1) were followed. Each individual cell was counted, using a microscope adjusted to give a factor of 600,000 and counting the number of fields designated by Standard Methods for the various count ranges.

TABLE 7  
Relationship of 37° C. plate counts and direct microscopic counts on 117 samples of ice cream

Plate count per ml.	No. of samples with direct microscopic count per ml. of					Total samples
	Less than 100,000	100,000-300,000	310,000-1,000,000	1,010,000-3,000,000	3,100,000-10,000,000	More than 10,000,000
Less than 1,000 .....	1	3	.....	.....	.....	.....
1,000-3,000 .....	5	1	.....	.....	.....	.....
3,100-10,000 .....	17	6	4	.....	.....	1
10,100-30,000 .....	13	2	6	1	.....	.....
31,000-100,000 .....	13	5	3	2	.....	1
101,000-300,000 .....	2	5	5	.....	.....	.....
301,000-1,000,000 .....	1	1	4	.....	1	3
1,010,000-3,000,000 .....	.....	.....	.....	2	1	2
3,100,000-10,000,000 .....	.....	.....	.....	.....	1	4
More than 10,000,000 .....	.....	.....	.....	.....	.....	1
Total samples .....	52	23	22	5	3	12
						117

TABLE 8  
Relationship of 45° C. plate count and direct microscopic count on 116 samples of ice cream

Plate count per ml.	No. of samples with direct microscopic count per ml. of					Total samples
	Less than 100,000	100,000-300,000	310,000-1,000,000	1,010,000-3,000,000	3,100,000-10,000,000	More than 10,000,000
Less than 1,000 .....	5	5	2	.....	.....	.....
1,000-3,000 .....	17	4	6	.....	.....	1
3,100-10,000 .....	17	3	3	2	.....	2
10,100-30,000 .....	11	2	5	1	.....	1
31,000-100,000 .....	3	6	2	.....	1	2
101,000-300,000 .....	.....	2	4	1	.....	3
310,000-1,000,000 .....	.....	.....	.....	.....	2	1
1,010,000-3,000,000 .....	.....	.....	.....	1	.....	1
Total samples .....	53	22	22	5	3	11
						116



## RESULTS

The data in table 7 indicate a tendency for parallelism between plate and direct microscopic counts, with the microscopic counts tending to be of somewhat greater magnitude than the plate counts. The latter is not unexpected, since individual bacterial cells, rather than "sources," were counted. By using a standard of direct microscopic count less than 1,000,000 per ml., only 6 of 97 samples below this standard had plate counts above 300,000 per ml. and none of these exceeded 1,000,000 per ml. in plate count; only 5 of the 20 samples exceeding this direct microscopic count limit had plate counts below 300,000 per ml., and 2 of these had such excessively high direct microscopic counts as to indicate the probability the plate count was at fault in evaluating the sample. A standard based on a requirement of a direct microscopic cell count of less than 1,000,000 per ml. would not have provided entirely satisfactory separation under the conditions of this study, although a microscopic count standard at this level might have definite possibilities in segregating the majority of samples of excessively high plate count. Clump or "source" counts were not made, as counts of individual cells seemed more desirable from a fundamental standpoint.

The data in table 8 indicate less relationship between the direct microscopic count and the plate count at 45° C. than between the direct microscopic count and the plate count at 37° C. Especially notable is the number of samples with comparatively low plate counts at 45° C. which have high direct microscopic counts. Since plate counts at 45° C. on ice cream appear to have little or no significance beyond those at 37° C., the comparative lack of relationship at 45° C. between plate counts and direct microscopic counts would appear to be of little significance in the evaluation of the latter method.

The data in table 9 show an almost complete lack of relationship between the direct microscopic counts and plate counts at 55° C.

## DISCUSSION

The ability of the direct microscopic method, counting individual cells, to detect most samples with high plate counts at 37° C. was demonstrated, as was its lessened usefulness in detecting organisms growing at 45° C. and its almost complete lack of suitability for detection of those bacteria favored by incubation at 55° C. Especially in view of the reportedly satisfactory use of the direct microscopic procedure for bacteriological control of pasteurized milk supplies in some areas (7, 9), the use of the method for control of certain types of ice cream supplies seems to be justified. The data do not indicate much possibility of use as a method of control of supplies on which low plate counts are the rule or for fine separation into several grades.

TABLE 9  
*Relationship of 55° C. plate count and direct microscopic count on 115 samples of ice cream*

Plant count per ml.	No. of samples with direct microscopic count per ml. of						Total samples
	Less than 100,000	101,000-300,000	310,000-1,000,000	1,010,000-3,000,000	3,100,000-10,000,000	More than 10,000,000	
Less than 1,000 .....	31	15	14	3	2	4	69
1,000-3,000 .....	9	3	4	.....	.....	3	19
3,100-10,000 .....	8	1	1	.....	1	1	12
10,100-30,000 .....	2	2	.....	1	.....	2	7
31,000-100,000 .....	2	2	1	1	.....	2	8
Total samples .....	52	23	20	5	3	12	115

## CONCLUSION

The direct microscopic count of individual cells offers possibilities as a screen test for detecting the bacteriologically poorer samples of ice cream, as indicated by the plate count at 37° C.

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# SULFUR COMPOUNDS AS DISINFECTING AGENTS FOR DAIRY EQUIPMENT\*

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## INTRODUCTION

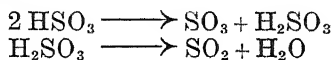
Chlorine compounds have been the most widely used germicidal agents employed by dairy plants and creameries. Activities incident to the prosecution of the World War, however, have created demands for chlorine compounds so extensive that supplies available for domestic use are substantially limited. This limitation may well become more drastic as the war continues. Consequently, it is necessary, as well as desirable, to find substances that can be substituted for chlorine. It was the purpose of the present investigation to study the disinfecting properties of possible substitute germicidal agents.

Sulfur was formerly thought to be a valuable disinfectant, especially in the form of sulfur dioxide. In recent years, however, experiments have indicated that this compound has limited disinfecting value. It is used effectively as a fungicide and vermicide, but its only practical use as a bactericidal agent has been in preserving dried fruits, and in this case its effectiveness appears to be due to the increase of hydrogen-ion concentration resulting from the formation of sulfurous acid.

Although there appeared to be little probability that sulfur compounds would prove to be of value for sterilizing dairy equipment, it seemed to be logical and necessary to obtain information about them, because there has been some revival of interest in their use as germicidal agents for dairy equipment. This report, therefore, is concerned with a study of the germicidal properties of certain sulfur compounds to determine their suitability for use in dairy plants.

## EXPERIMENTAL

Acid sulfites are of two types, the bisulfite and the pyrosulfite (meta bisulfite), the latter being the anhydride of the former. The pyrosulfites do not exist in solution, but they dissolve in water and ionize, yielding a cation and the anion, hydrogen sulfite, which is believed to undergo the following transformation:



Solutions of the sulfites gradually decompose with the separation of sulfur and the production of sulfuric acid. As sterilizing agents their solutions are too corrosive for practical use, so it was necessary to buffer them.

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In the work reported here, 5 per cent solutions of potassium pyrosulfite were buffered at pH 6, 7, 8, and 9. The buffer systems employed were as follows: Citric acid and sodium hydroxide at pH 6, trisodium phosphate at pH 7, and boric acid and trisodium phosphate at pH 8 and 9. On this basis the free sulfur dioxide produced amounted to 45 to 50 per cent of the weight

TABLE 1  
*Sterilizing properties and corrosive action of various sulfur compounds*

Substance	Concentration used*	pH value	Survival of bacteria after varying periods of contact			Corrosive action on tin	
			5 min.	10 min.	15 min.	Loss (-) or gain (+) in weight	Remarks and general appearance
Potassium meta bisulfite .....	1.0%	4.5	%	%	%	%	
“	1.0%	7.2	5.0	4.0	5.0	—	Very corrosive immediately
“	5.0%	6.0	24.0	30.0	23.0	—	Sl. corrosive after 4 weeks
“	5.0%	7.0	12.0	10.0	12.0	6.68 -	Visible corrosion
“	5.0%	8.0	12.0	15.0	13.0	0.028 -	Not tarnished
“	5.0%	9.0	10.0	12.0	10.0	0.006 -	Not tarnished
“	5.0%	9.0	12.0	9.0	9.0	0.021 -	Not tarnished
“	10.0%	4.0	2.0	2.0	1.0	—	—
“	10.0%	7.2	27.0	29.0	35.0	—	—
Sulfur dioxide.....	1.4%	1.6	0.0	0.0	0.0	—	—
“ “ .....	2.8%	1.4	0.0	0.0	0.0	—	—
“ “ .....	5.6%	1.3	0.0	0.0	0.0	—	—
“ “ .....	11.0%	—	0.0	0.0	0.0	—	—
Acid sodium sulfite .....	1.0%	—	29.0	29.0	30.0	—	Visible corrosion
Sulfuric acid .....	—	1.4	0.08	0.08	—	—	Visible corrosion
H.T.H. 15 .....	200 ppm. Cl	.....	0.0	0.0	0.0	0.002 +	Not tarnished
Distilled water ...	—	6.4	—	—	—	0.015 +	Not tarnished

\* Percentages are by weight.

NOTE: The dashes indicate that no determinations were made because, in comparison with weaker or less acid concentrations, results would be obvious. This statement does not apply to H.T.H. and distilled water.

of the pyrosulfite, which is equivalent to a 2.25 to 2.50 per cent solution of sulfur dioxide. In all analytical work, reagent grade sodium bisulfite of known sulfur dioxide content was used as a control.

When dairy equipment is being sterilized with chemical agents, the milk film that is likely to remain in and on the equipment may combine with the agent and then partially inactivate it. It is well known that many disinfectants, chlorine included, can be inactivated in this way. For that reason, milk was combined with the sulfur compounds employed. The milk also

provided a source of bacteria for testing the disinfectant properties of the compounds.

The sterilizing properties of the various solutions were determined by adding 2 ml. of raw milk to 48 ml. of the solution to be tested. Several different lots of raw milk were employed. Immediately after the milk was added, each container was shaken rapidly 25 times, each shake being an up-and-down excursion of about a foot. Proper dilutions were made, and 1 ml. quantities were plated after the milk had been in contact with the solution being tested for 5, 10, and 15 minutes. Standard tryptone-glucose-extract agar was used, and the plates were incubated for 48 hours at 37° C. The concentration of inoculum (ranging from 65,000 to 12,000,000 per ml.) was determined by adding 2 ml. of milk to 48 ml. of sterile distilled water and plating by the dilution method. A chlorine solution (H.T.H.) containing 200 ppm. of available chlorine served as a control. Milk was added and the same procedure employed as with the test solutions.

The corrosive properties of the sulfur compounds were tested on tinned copper strips of 1 inch by 2.5 inches dimensions. The metal strips were polished, washed, dried, and weighed, and then were immersed in 125 ml. of the various solutions, which were agitated at room temperature. After 16 hours, the metal strips were removed from the solution, rinsed with distilled water, dried, and reweighed to determine any loss or gain in weight. The general appearance of the metal strips was also noted to observe any obvious evidence of corrosion. In a few instances the solutions were not agitated, and the metal strips remained in the solutions at room temperature for four weeks.

The results of the bacteria counts and of the corrosion tests are shown in table 1. It should be noted that in all instances where the bacteria counts were reduced sufficiently to encourage the use of the agent, the pH values were quite low. (Although the pH value is not given for 11 per cent sulfur dioxide, it was unquestionably lower than for the other concentrations employed.) Thus, it would appear that any substantial disinfecting property displayed was due to increased hydrogen-ion concentration. Above pH 6, the percentage reduction of bacteria was not sufficient in any instance to justify recommending the use of any of the agents employed.

#### CONCLUSION

The results of the experiments here reported do not encourage the use of sulfur compounds for dairy plant sterilization.





# LIVE-WEIGHT GAINS OF PASTURE-FED DAIRY HEIFERS

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It is well known that size of cow bears a close relation to milk-producing capacity. Large size is best obtained through programs of feeding and management which will produce rapid live-weight gains throughout the developmental period prior to first calving. While pasture feeding is one of the best ways of inducing good growth in heifers, pasture forage yields vary greatly from farm to farm, depending upon kind of pasture crop and fertility of the soil, and from month to month, or season to season, depending upon rainfall and temperature. The live-weight gains of heifers having access to pasture as the only feed are likely to follow the same trends as the pasture forage yields and may be positive or negative.

How much live-weight gain do dairy heifers make when pasture is the only source of feed? Do the dairy breeds differ in their ability to utilize pasture for growth? But few reports of experiments which supply answers to these questions are to be found in the literature. Bender and Bartlett (1) observed losses in weight of 40 to 100 pounds per head during the first month after fat two-year-old heifers were turned to pasture, while heifers winter-fed on roughage suffered no loss in weight and made greater total gains for the season. Eckles (2) made comparisons of the efficiency of Holstein and of Jersey heifers in the use of alfalfa hay only and a combination of alfalfa hay and corn silage for the making of winter live-weight gains. The Holsteins consumed more feed, made larger daily gains, and required less feed per pound of gain. Hayden (3) found that 29 Jersey heifers, one to two years of age, which were kept at pasture for 159 days, made daily gains for the year equivalent to 0.8 pound per head; while 22 Holstein heifers of the same age, which were pastured 151 days, made daily gains during the year amounting to 1.0 pound per head. Henderson and Anthony (4) reported daily live-weight gains of yearling dairy heifers kept at pasture from 194 to 203 days as ranging from 0.3 to 1.13 pounds per head. In a farm survey conducted by Misner (5), it was found that the gain in weight of heifers in 107 dairy herds, the majority of which were grade Holstein, amounted to 261 pounds during the period from 12 to 24 months of age. The heifers were pastured for approximately 169 days.

## EXPERIMENTAL PROCEDURE

The records of 178 dairy heifers which had access to pastures, water, and salt as the only sources of feed during approximately five months of the year form the basis of the present study. The records covered the years 1936 to

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1943, inclusive. The ages at the beginning of the pasture season ranged from 8 to 24 months. None of the heifers calved or aborted while at pasture. They were turned to pasture in late April or early May, and kept on pasture until about October 1. In seasons of low rainfall (1937 and 1940) the pasture season was shortened, but in 1941 abundant rains made possible the use of pastures until the third week of October. The length of the pasture season ranged from 112 to 170 days. Scale weights were taken on three successive days just prior to turning to pasture and at 4-week intervals thereafter.

In 1936 and 1943, the heifers were pastured as one group, but in other years there were two or three groups of heifers employed in a comparison of different pasture crops. Differences also occurred in the number of heifers of each breed used in these comparisons. These differences are considered of too little importance to nullify or seriously impair the worth of the data as a basis for the present study since the heifers had essentially the same opportunities of obtaining ample amounts of feed. The experimental pastures were carefully managed and usually provided abundant forage.

#### DISCUSSION OF RESULTS

The average live-weight gains ranged from approximately two-thirds pound daily for the Brown Swiss heifers to approximately one pound daily for the Ayrshire, with an average of 0.84 pound per head daily for all heifers (table 1). Sometimes the rate of daily live-weight gain is used as the sole criterion of the results of feeding trials. On the basis of live-weight gains alone, it appears in the trial here discussed that the Ayrshires and Holsteins were superior to the other three breeds. There are other pertinent criteria, however, which should be given attention when evaluating such data. One of these is the daily gain in relation to the initial live weight. It is shown in table 1 that the Ayrshires, Guernseys, and Jerseys, made approximately the same live-weight daily gains when these are expressed as a percentage of the initial weights. These values are higher than those for the Brown Swiss and Holsteins.

A further criterion of the grazing ability of the breeds is the live-weight gain in relation to the amount of body weight maintained. The average of all scale weights for the season is assumed to be the amount of body weight maintained. The Holsteins maintained about 40 per cent more body weight and also made slightly larger daily gains than the Guernseys and Jerseys. This implies that the Holsteins consumed much more feed per head daily than heifers of the Guernsey and Jersey breeds. By the same reasoning, it is inferred that, with smaller amounts of pasture forage consumed and nearly as large daily gains per head made by the Guernseys and Jerseys, gains by these breeds were more economical. If the pastures were fully stocked, more Guernseys and Jerseys might be supported per acre of pasture, and the gains in live weight per acre would be larger.

A study was made of the variance of the daily gains of the heifers of each breed and the mean differences in daily gains of the five breeds. Using the  $F$  test as given by Snedecor (6) the calculated  $F$  value for the data is only 1.86, whereas values of 2.43 at the 5 per cent level, and 3.44 at the 1 per cent level, are required for significance. Applying the  $t$  test as stated by Snedecor, it is calculated that a difference of 0.437 pound would be required to show a significant difference in daily live-weight gains between any two of the breeds. It appears, therefore, that under the conditions of this trial,

TABLE 1

*Live-weight gains of yearling dairy heifers with pasture as the only feed*

	Average values per head				
	Ayrshire	Brown Swiss	Guernsey	Holstein	Jersey
Number of records .....	27	12	26	79	34
Age at beginning of pasture season, days .....	495	495	453	483	470
Live weight at beginning of pasture season, lbs. ....	696	823	565	832	564
Average live weight for entire pasture season, lbs. ....	771	882	623	891	625
Number of days on pasture .....	155	151	147	142	151
Total live-weight gain for season, lbs. ....	151	103	113	123	120
Live-weight gain daily, lbs. ....	0.97 $\pm$ 0.03	0.68 $\pm$ 0.05	0.77 $\pm$ 0.01	0.86 $\pm$ 0.03	0.80 $\pm$ 0.04
Standard error of mean .....	0.26 $\pm$ 0.02	0.23 $\pm$ 0.03	0.30 $\pm$ 0.03	0.38 $\pm$ 0.02	0.36 $\pm$ 0.03
Coefficient of variability of daily live-weight gains .....	26.95 $\pm$ 0.10	33.89 $\pm$ 0.67	39.62 $\pm$ 0.11	43.73 $\pm$ 0.06	36.67 $\pm$ 0.09
Daily live-weight gain as percentage of initial weight, lbs. ....	0.139	0.083	0.136	0.104	0.141
Relative amounts of live weight maintained .....	87	99	70	100	70

no significant differences were revealed in the inherent ability of these five breeds to make daily live-weight gains while pasture fed. This does not preclude the possibility that an experiment designed especially to study this point might reveal such differences.

#### SUMMARY AND CONCLUSIONS

Live-weight records of 178 pasture-fed yearling heifers of the Ayrshire, Brown Swiss, Guernsey, Holstein, and Jersey breeds made during the years 1936 to 1943, inclusive, show average daily live-weight gains for the entire group of 0.84 pound daily. Average daily live-weight gains for the breeds ranged from 0.701 pound for the Brown Swiss up to 0.963 pound for the

Ayrshire. In terms of percentage of the initial live weights, the average daily gains of the Ayrshires, Guernseys, and Jerseys were approximately the same and were somewhat higher than the corresponding values for the Brown Swiss and Holsteins. Statistical analyses of the variance of the live-weight gains indicate that in this trial no significant superiority of any breed to make live-weight gains while pasture fed was demonstrated.

Because of characteristic differences in body size, it is assumed that some breeds made live-weight gains with less pasture forage than others because of using less feed for maintenance. For example, the average daily live-weight gains of the Guernseys and Jerseys were nearly as large as those of the Holsteins, but the average live weight of the Guernseys and Jerseys was about 30 per cent less.

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## THE USE OF UREA IN COMMERCIAL DAIRY FEEDS

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In November, 1943, a small amount of crystal urea was made available under allocation to feed manufacturers. Previous to that time most of the urea not used for the war industries was allocated to the fertilizer trade. For several years the experiment stations in the large dairy states have been getting urea for research use; and in 1941 the Association of American Feed Control Officials, foreseeing its feeding use, adopted a resolution accepting urea as an ingredient in proprietary cattle feeds.

Hart *et al.* (2), in 1939, studied the utilization of urea by growing calves. They found that the conversion of urea nitrogen to protein was limited and dependent on the total protein in the ration. Mills, Booth, Bohstedt and Hart (5) in 1942 found that the micro-organisms responsible for the use of non-protein nitrogen needed an available source of energy; that starch was more available as a source of energy than timothy hay. Rupel, Bohstedt and Hart (6) found that urea should not be fed at a rate greater than 1 per cent of the dry matter of the ration. This amounts to about 25 per cent urea nitrogen in the entire ration.

Loosli and McCay (4) found that calves as young as two months were able to grow on a ration containing about 75 per cent of the total nitrogen from urea. Johnson *et al.* (3) found that for sheep the biological value for urea was about 62.

Goss (1) gives a good discussion of the mechanism involved in converting urea to protein in the ruminant. He concludes that urea may replace part of the protein in basal diets containing insufficient protein to maintain nitrogen equilibrium, and adds that considerable work must be done before optimum conditions for the use of urea are understood.

Mills *et al.* (5) state "the effect of various ration constituents on the efficiency and rate of urea utilization should be thoroughly studied so that when and if urea becomes a practical nitrogen source for the farmer, urea-containing rations could be intelligently constructed to obtain the best results with as little waste as possible."

The protein shortage which developed in 1943 made it advisable to use urea before all the questions had been answered. The work reported here was undertaken in an effort to further the data on the use of urea as it is used in commercial dairy rations. The part of the work completed so far reports on the effect of urea on milk production, milk composition, palatability of feed and general health of the cow.

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Of the herds available to us for a feeding test, there was one that was outstanding. It consisted of about 30 registered Holsteins owned by a small local college. Last year, the third consecutive year on official test, the herd average was 437 pounds of butterfat and 12,933 pounds of milk. The herd is under the constant care of a dairy manager and has been free from tuberculosis, brucellosis and mastitis.

Seven cows in one section of the milking barn were chosen for the test group. It was judged unwise at that time to place more than this number on a ration that might give unfavorable results. The cows in this group were near the herd average in age and previous production, and had freshened between September and November, 1943. The rest of the herd received the control ration. Fifteen cows near the herd average of production and

TABLE 1  
*Dairy ration grain concentrate formulæ*

Ingredients	Control ration	Test ration						
		Nov.	Dec.	Jan.	Feb.	March	April	May
Corn meal .....	400	400	400	400	400			
Wheat bran .....	250	250	250	250	250			
Distillers' grain .....	400	400	400	400	300			
Corn gluten feed .....	350	200	100					
Crimped oats .....	200	200	200	200	200			
Yellow hominy .....		310	400	490	580	Same	Same	Same
Crystal urea .....		35	42	50	60			
Soybean oil meal .....	200							
Molasses .....	140	140	140	140	140			
Limestone .....	20	20	20	20	20			
Bonemeal .....	20	25	28	30	30			
Iodized salt .....	20	20	20	20	20			
Total .....	2000	2000	2000	2000	2000	2000	2000	2000

age, freshening between September and November, were chosen for record comparisons. The cows were milked twice a day and the milk weighed. All silage, hay and grain concentrates were weighed at each feeding.

Table 1 shows the formulæ for the grain concentrate used in the test and control rations. The average chemical composition is 20.6 per cent protein, 4.5 per cent fat, 7.2 per cent fibre. The whole herd has been on the control ration since October, 1941. On November 1, 1943, the test group was given a mixture in which 35 lbs. urea, 310 lbs. hominy and 5 lbs. bonemeal replaced 200 lbs. soybean oil meal and 150 lbs. corn gluten feed. This kept the levels of protein ( $N \times 6.25$ ), fat, fibre and total digestible nutrients the same as in the original ration. In December, 100 pounds of corn gluten feed was omitted from the test ration; and 7 pounds urea, 3 pounds bonemeal and 90 pounds of hominy were added. On January 1, 1944, the entire 350 pounds of gluten feed was omitted, with urea and hominy feed making up the difference in protein and other nutrients. This made the non-protein nitrogen 36 per

cent of the total. On February 1, 100 pounds of distillers grains was omitted, with urea and hominy added to make the non-protein 43 per cent of the total. The test group has continued on this ration up to the present time.

#### UREASE ACTIVITY IN SILAGE

The feeding plan for this herd is quite common to most farms in this section of the country. Cattle are on pasture or in the "tramp" shed except during the milking period. In the early part of the afternoon, when most of the help is available, silage is weighed out and put into the manger. Then the grain concentrate is weighed out and placed on top of the silage. In the middle of the afternoon the cows come into the milking barn and to their stanchions. The question was raised as to whether urea in the grain concentrate would be stable during the one- or two-hour period in contact with the silage.

Samples of different silages were brought into the laboratory and tested for urease activity. Soybean, alfalfa and corn silages were put into jars with urea and kept under practical feeding conditions. Water was added to some of these samples. An aliquot portion was taken from the jars at intervals and tested for free ammonia. When water was added and the material incubated at 37° C., ammonia was detected in a few hours. However, it took several days at ordinary room temperature with the original moisture content of the silage before ammonia was evident in the samples.

#### PALATABILITY OF FEEDS

During the seven months that urea has been available for dairy feeds, we have had some complaints of feed refusal and digestive disorders among cows on urea-containing feeds. Each of these complaints was investigated with the thought in mind that urea might be causing this trouble. Each case, however, was due to some other condition. Sometimes in returning to the milking ration from a fitting ration the cows refused the feed and remained off feed even when presented with a ration not containing urea.

With the herd on test no trouble with palatability or off feed was experienced during the entire period. On a group of six cows the feed was changed overnight, and each time the test ration or the control ration was consumed with equal relish. It is a well-known fact that Holsteins are not as particular in their feeding habits as Guernseys or Jerseys. A herd of Guernseys receiving a commercial ration containing no molasses was tested for its reaction to urea. Three per cent urea was added to this dried ration and fed for four days. No cow in the herd showed any sign of refusing the feed.

#### MILK COMPOSITION

The butterfat content of the milk in the herd under consideration was tested each month by the official tester for the Dairy Herd Improvement

TABLE 2  
Monthly record of milk production and butterfat percentage

	October		November		December		January		February		March		April		May	
	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat
	lbs.	%	lbs.	%	lbs.	%	lbs.	%	lbs.	%	lbs.	%	lbs.	%	lbs.	%
Harvel	32	3.7	40	3.6	37	3.6	31	3.2	30	3.0	27	3.4	27	3.0	28	3.3
Henny	43	3.2	43	3.4	39	3.9	35	2.9	34	3.0	33	2.9	32	2.9	35	2.9
Heath	40	3.8	53	3.4	51	3.7	44	3.2	41	3.1	37	3.2	35	2.9	29	3.5
Hara	39	2.9	37	3.5	31	3.3	28	3.5	24	3.2	23	3.3	23	2.8	23	3.9
Harley	43	3.6	44	2.9	40	3.1	35	3.6	33	3.7	28	3.5	24	3.6	19	3.0
Hue	Dry	.....	Dry	.....	51	3.0	53	3.0	51	2.9	46	3.2	46	2.8	45	3.6
Heuton	46	3.1	43	3.0	39	3.2	35	3.1	32	3.3	29	3.1	28	3.2	25	4.4
Average	40.5	3.3	43	3.3	41	3.3	38	3.2	35	3.2	32	3.2	31	3.2	29	3.5
Hofer	39	3.3	37	3.5	35	3.6	32	3.4	29	4.0	27	4.0	29	3.9	27	3.7
Hat	53	3.8	48	3.6	42	3.5	39	3.2	37	3.2	34	3.2	32	3.5	28	3.1
Hackle	Dry	.....	41	3.7	52	3.6	50	3.3	46	3.7	38	3.1	41	2.7	38	3.5
Hab	Dry	.....	54	4.0	52	3.7	47	3.3	44	3.4	43	3.4	43	3.3	39	4.0
Hoylton	Dry	.....	Dry	.....	59	3.3	55	3.0	51	3.2	48	3.0	45	2.9	46	3.2
Helmar	Dry	.....	Dry	.....	34	3.3	33	3.2	29	3.2	26	3.1	25	3.0	24	3.1
Hucker	36	3.0	39	3.1	35	3.2	32	3.2	30	3.1	28	3.2	29	2.7	28	3.6
Helag	40	3.3	40	3.7	35	3.5	34	3.4	34	3.4	23	3.3	33	3.3	32	3.6
Hattag	37	4.2	37	3.6	35	3.0	31	3.1	30	3.3	28	3.4	27	3.0	26	3.7
Harlag	Dry	.....	Dry	.....	31	3.9	30	3.7	29	3.5	28	3.6	27	3.4	29	3.1
Hamill	38	3.2	40	3.3	34	3.2	32	3.4	32	3.3	31	3.7	34	3.2	34	3.5
Helma	31	3.1	28	3.4	25	3.0	23	3.1	21	2.7	20	3.0	20	3.7	16	4.3
Henning	37	3.3	35	3.3	37	3.8	33	3.3	34	3.9	19	3.8	18	3.3	18	4.1
Hika	45	3.0	44	3.6	39	3.8	34	3.8	30	3.5	27	4.0	26	3.5	25	3.1
Heart	48	3.1	48	2.9	42	3.1	34	3.0	38	3.1	34	3.0	32	2.8	28	3.7
Average	40.4	3.4	41	3.3	39	3.4	36	3.3	34	3.4	31	3.4	31	3.3	29	3.6



Association. The percentage of butterfat in the test group averaged 3.3 per cent during the test period. The percentage of butterfat in the control group averaged 3.4 per cent.

Three times during the test period protein tests were made on milk taken from the different groups. Each time the average milk protein in the test group was slightly higher than that in the control group, although individual tests varied by more than this difference. The overall average for milk protein in the test group was 3.2 per cent, with that of the control group 3.0 per cent.

#### MILK PRODUCTION

The cows were milked twice a day and each milking was weighed to the nearest tenth of a pound. Average production for each cow during each month of the test is tabulated in table 2. The average per cow for the test group and the control group is very nearly the same for each month over the test period. The average for the seven months is 36 pounds per day for the test group and 35 pounds for the control group.

#### DISCUSSION

There is not much question that, over the short period of this test, urea can be used in dairy rations to replace part of the natural protein without unfavorable results. Milk production has been maintained in the test group at a level equal to that in the control group. Milk fat and milk protein in the test group agree with that in the control group within the limits of probable error. The results have been summarized in table 3.

TABLE 3

*Average daily milk production, milk composition and feed consumption on the test and control rations*

	Test ration	Control ration
Number of cows fed .....	7	15
Number of days in period .....	210	210
Daily feed, pounds		
Concentrates .....	10.8	10.5
Hay .....	10.0	10.0
Silage .....	21.4	21.4
Daily milk, pounds .....	36.0	35.0
Per cent of fat .....	3.3	3.4
Per cent of protein .....	3.2	3.0
Weight, pounds—April .....	1443	1382
June .....	1471	1411
Gain .....	28	29

We do not know yet what these results may be over a longer period of time. The feeding requirements at a given level of production can be calculated. For example, the cows on test producing an average of 36

pounds of milk a day, with an average protein content of 3.2 per cent, would need 1.15 pounds of protein. Add to this the protein requirement for maintenance, which is 0.9 pounds a day for cows of this weight, and you get a total daily requirement of 2.05 pounds of protein. By using Morrison's figures of protein digestibility, these cows received 2.3 pounds of digestible protein a day. However, if urea were not available as a source of protein or if the protein intake per day were figured without urea, each cow would receive 1.6 pounds a day digestible protein. This would leave the cow deficient to the extent of about 0.5 pound protein a day. It would not take long at this level for the cow to show some evidence of deficiency, either in decreased production or in loss of weight. Of course, the accepted figures for maintenance over a short period may be considerably lower than now estimated, but it would have to go down to 0.3 pound digestible protein a day per 1000 pounds weight, before this deficiency would be corrected.

One of the cows in the test group aborted a five-month-old fetus. The reason for this could not be determined. A veterinarian's report on the herd showed normal embryonic development and weight<sup>1</sup> between April and June. The cows in each group had maintained milk production and gained an average of 28 pounds during these two months.

The whole herd on test did not give as good results as the previous years' records would indicate. One reason for this is that the cows were milked only twice a day, whereas three milkings per day had been the practice in past years. Another reason may be the low quality of hay available. The alfalfa stand had been overrun with orchard grass, giving the hay a high fibre content and a lower T.D.N. value. These tests are continuing and will include records from all the cows in the herd, some of which have production figures of 23,000 pounds a year.

#### SUMMARY

1. Urease activity was observed in all silage samples tested; but under average conditions of time, temperature, and moisture there was no practical destruction of urea when added to a dairy ration and placed over the silage in the manger.

2. No condition of unpalatability or digestive disorder in any commercial or test ration containing urea could be traced to the use of this ingredient.

3. The percentage of butterfat and protein in the test group and in the control group did not differ significantly.

4. Milk production during the first seven months of lactation was the same in the test group as in the control group.

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<sup>1</sup> Weight was estimated by means of the chest measure.

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### BOOK REVIEW

1. **Food Enough.** JOHN D. BLACK. The Jaques Cattell Press, Lancaster, Pa. 1943. \$2.50.

In addition to including basic facts on the need for food and food nutrients, this book analyzes the world's food supplies and the nutritional status of various peoples, points out the problems of war time feeding, considers production of food on the basis of most effective utilization of natural resources, suggests policies that would best cope with the present food and feed crisis, and recommends a course of marketing procedure designed to overcome much of the difficulties arising between the points of production and ultimate consumption.

Included also are excellent discussions of the need for and mechanics of rationing, shifts in dietary patterns, industrial feeding, and the world-wide significance of the United Nations Conference on Food and Agriculture.

The author is Henry Lee Professor of Economics at Harvard University, a member of the Economic Panel of the Interim Commission of the United Nations Food Conference, and a member of the Food and Nutrition Board of the National Research Council.

The importance of food and the relationships between food supplies and the course of world events now and in the future make this volume of great timeliness. Written by an economist with much practical experience and with many associations that place him in a position to know the facts, and written in a style that is easily read by anyone, *Food Enough* should be on the "must" list for all. Agriculture in general and dairying in particular are given encouraging places in future world economy. W.E.Krauss.

### BACTERIOLOGY

2. **Bacteriophages for *Streptococcus cremoris* Phage Development at Various Temperatures.** G. J. E. HUNTER, Dairy Res. Inst., Palmerston North, New Zealand. Jour. Dairy Res., 13, No. 2: 136-145. 1943.

The effect of temperature on the growth in milk of several strains of *Streptococcus cremoris* and their appropriate phages was investigated. Acid formation and rate of phage multiplication were followed in 100-ml. quantities of autoclaved skimmilk seeded with 1% of an 18-20-hour clotted-milk culture and varying amounts of phage added. At intervals 2 ml. were withdrawn for acidity titration and 1 ml. was used for dilution in the plate test for phage multiplication.

In general, the streptococci grew more rapidly at 30° C. (86° F.) than at 22° C. (71.6° F.) but were inhibited to a greater or less degree at 37° C. (98.6° F.). The phage races for the most part developed somewhat more readily at 30° C. (86.0° F.) than at 22° C. (71.6° F.). Some developed just as readily at 37° C. (98.6° F.) as at 30° C. (86° F.) but others were completely inhibited at 37° C. (98.6° F.).

These results suggest that the phage races as they exist are separate and distinct entities whose growth conditions are similar to, but not necessarily identical with, those of the bacteria upon which they develop. S.T.C.

3. Distribution of *Flavobacterium maloloris*. R. M. REYNOLDS AND H. R. THORNTON, Univ. of Alberta, Canada. Sci. Agr., 24, No. 1: 21. 1943.

Workers in this laboratory had previously found *F. maloloris* to be an infrequent cause for surface taint in butter. In this study 589 isolations of yellow bacteria from 140 samples of Alberta water were examined and only two proved to be *F. maloloris*. O.R.I.

4. The Occurrence of Slow-Reducing Coliform Organisms in Milk. C. S. MORRIS, Seale Hayne Agr. Col., Newton Abbott, Devon, England. Jour. Dairy Res., 13, No. 2: 115-118. 1943.

Milk samples were found frequently to contain coliform organisms which give very slow reduction of methylene blue at 37° C. (98.6° F.). Evidence was secured which indicates that this slow reduction is due to two factors present in raw milk, both of which are destroyed by heating milk to 70° C. (158° F.) for one hour. These factors are (a) a specific bactericidal substance and (b) a growth-inhibiting factor.

All of the cultures examined appeared from differential tests to be intermediate types of coliform organisms. All were citrate negative and failed to produce acid or gas when grown in MacConkey's broth at 44° C. (111.2° F.). S.T.C.

## BUTTER

5. Churn Washing Procedure. S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., 5, No. 2: 120-122. 1943.

Dry rot of staves of the churn barrel is caused by fungi which could be destroyed by heat if it were possible to sufficiently heat all parts, around bolts, crevices, and corners. Cypress is less susceptible to dry rot than Douglas fir. The treatment of churns with hot water apparently neither weakens the wood nor favors dry rot. The most satisfactory procedure of washing the churn appears to be, first, a rinse with water at 120-140° F. to remove the bulk of the fat; second, running the churn for 15 minutes half full of water at 180° F. containing a mild alkaline washing powder; and,

third, running the churn for 15 minutes half full of water at 200° F., draining thoroughly, and allowing to dry with the doors open. Reduction in the temperature of the water does not accomplish the purpose or prolong the life of the churn. P.S.L.

6. **Water Supply Difficulties.** S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., 5, No. 2: 46-48. 1943.

Where raw water used as wash water for butter causes off flavors due to bacterial contamination and chlorinated water does the same due to its chemical content, the ideal procedure is to dechlorinate the water before use. The ultra violet ray water sterilizer is of little value for this purpose. The use of small amounts of chlorine causes more off flavor due to chlorophenol and similar compounds than does large amounts of chlorine. Many creameries successfully use wash water containing 25 p.p.m. of chlorine. The writer describes a method for construction of a dechlorinating filter.

P.S.L.

7. **Court Cases Involving Butter.** LEO T. PARKER, Attorney at Law, Cincinnati, O. Amer. Butter Rev., 5, No. 1: 8, 10, 12. 1943.

The author lists several legal interpretations, decisions, and rulings concerning butter; the validity of state laws; public policy, monopoly, trade mark, and ceiling price laws; and validity of contracts. Each is illustrated with recent court decisions.

P.S.L.

8. **Moisture Loss in Prints—Combatting Leaky Bodied Butter.** S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., 5, No. 3: 86. 1943.

Due to reduced water retaining powers winter butter moisture standards should be reduced if leakiness is to be prevented. Such procedure as reduces size of the water droplets helps in reduction of leakiness. These include working the butter while firm, use of 40° F. wash water, filling the churn no more than 40% full, prevention of stickiness of the churn, and adding all water of standardization at the time of adding the salt.

P.S.L.

## CHEESE

9. **Bacteria, Environment and Cheese.** E. G. HASTINGS, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., 34, No. 10: 14. Oct., 1943.

Its environment determines the fate of every living thing—although man cannot always recognize how it does so. The cheesemaker seeks to establish in milk, curd and cheese a desired bacterial pattern by controlling tempera-

ture, acidity, moisture and salt. His sensitivity to the milk is most important in the making of uniform cheese. The uniformity expected by many is unattainable but can be best achieved by the use of milk from the same farms daily, to the bacterial pattern of which the maker has adapted himself. A change of milk or maker can be expected to change quality. Pasteurization of milk simplifies the bacterial pattern and though it slows flavor development it may be possible to find ripening agents to remedy this effect. If normal, three or four months old pasteurized-milk cheese is ground and stirred thoroughly, then flavor development approximates that in raw-milk cheese. The few essential, flavor-producing bacteria which survive pasteurization are scattered by this mixing, so that they reach the food they need and quickly produce flavoring substances. Ground cheese can also be blended for uniformity and reformed and packaged if provision is made for the venting of CO<sub>2</sub> formed by the continued action of ripening agents. Occasionally pasteurized-milk cheese cures normally without CO<sub>2</sub> development which suggests new ripening possibilities if it were understood. Too little attention has been given to producing the smooth, waxy body desired by most consumers; too much attention has been given to eye formation in Swiss. "Eyes in a woman or a Swiss cheese are something to consider but not to control one's judgment as to desirability." W.V.P.

10. **The Effect of Over-Ripening upon the Activity of Cheddar Cheese Starters.** C. K. JOHNS AND H. L. BERARD, Div. of Bact. and Dairy Res., Sci. Serv., Dept. Agr., Ottawa, Canada. *Jour. Dairy Res.*, 13, No. 2: 127-135. 1943.

In a study involving three starters, over-ripening brought about by increasing the amount of inoculum, by lengthening the period of incubation, or by increasing the temperature of incubation, failed to slow down the rate of bacterial growth, or acid development, or to lower the final acidity reached. Starters repeatedly over-ripened, over a period of 30 days were considered superior in flavor to those normally ripened.

In practical cheesemaking an over-ripened portion of a starter worked slightly faster in the vat and produced cheese with a higher flavor score than the control. S.T.C.

11. **The Influence of Abnormal ("Non-Acid") Milk on Cheese Starter Cultures.** G. J. E. HUNTER AND H. R. WHITEHEAD, Dairy Res. Inst., Palmerston North, New Zealand. *Jour. Dairy Res.*, 13, No. 2: 123-126. 1943.

"Non-acid" milk is described as a colloquial term coined by cheesemakers in New Zealand to designate milk which hinders the development of acid by a normally active starter culture in the cheese vat. Stock cultures of "non-acid" organisms were grown at 30° C. (86° F.) in sterilized skimmilk for

24 hours. The acidity rise was 0.03–0.06 per cent expressed as lactic acid. This “non-acid” skimmilk was mixed in varying proportions with normal fresh milk and the resulting mixture used for starter propagation. Delayed coagulation simulating a starter failure caused by bacteriophage was demonstrated. Some strains of starter cultures were considerably less inhibited than others. S.T.C.

12. Further Studies on Bacteriophage in Relation to Cheddar Cheese-making. C. K. JOHNS, Div. of Bact. and Dairy Res., Sci. Serv., Dept. Agr., Ottawa, Canada. Jour. Dairy Res., 13, No. 2: 119–122. 1943.

Trouble with slow working vats in two factories which had continued over a period of six weeks was shown to be due to lysis of the dominant strains of the mixed culture of starter organisms by phage. Normal acid development was secured by substitution of a starter of entirely different bacterial strains.

A second outbreak of phage infection with complete cessation of acid production in experimental vats is described. The starter itself appeared to be entirely free from phage and the equipment had been drastically sterilized following a previous outbreak. The original source of the phage was not definitely demonstrated although occasional positive indications were obtained from the milk supply itself. S.T.C.

13. The Production of Rennet from Living Calves. N. J. BERRIDGE, J. G. DAVIS, P. M. KON, S. K. KON, AND F. R. SPRATLING, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. Jour. Dairy Res., 13, No. 2: 145–161. 1943.

Abomasal juice containing rennin was obtained from living calves by means of an abomasal fistula. The operation for fistula was performed on two calves at 14 days of age. The animals received, during the three months for which the experiment lasted, an exclusive diet of whole milk supplemented by minerals and vitamin D.

Abomasal juice was obtained by allowing the calves to drink dilute whey and removing it through the fistula in about half an hour. The mean yield of rennet for each “perfusion” from the first calf was 3120 units with a standard deviation of  $\pm 1330$  and 5680 units with a standard deviation of  $\pm 2560$  from the second calf. One unit of rennet is defined as the amount sufficient to coagulate in 100 seconds 10 ml. of a substrate consisting of 12 grams of spray-dried skimmilk in 100 ml. of N/50 calcium chloride solution.

A concentrated rennet was prepared from the abomasal juice. Cheese made from the fistula rennet was indistinguishable from the control cheese.

This method of rennet production was regarded as too expensive in time, labor, and cost of food for use in commercial rennet production in England. S.T.C.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

## 14. Relation of Lecithin to the Keeping Quality of Dry Whole Milk.

C. D. DAHLE AND D. V. JOSEPHSON, Pennsylvania State College.  
Natl. Butter and Cheese Jour., 34, No. 10: 18. Oct., 1943.

Fresh milk was separated into cream and skim milk. The cream was churned and the butter melted and washed to obtain pure butter oil. A portion of the skim was centrifuged at 25,000 RPM to reduce the lecithin in the skim. Samples of the fresh milk, pure butter oil and skim, and pure butter oil and centrifuged skim were pasteurized, homogenized, concentrated 2 to 1, and dried by the atmospheric roll process. The dry milk was placed in brown bottles. Half of each lot was charged with nitrogen, and all containers were carefully sealed and stored at 85° F. Some of each was re-constituted and examined at monthly intervals. The samples with least lecithin kept best although all samples deteriorated. Over half of the milk lecithin remained in the skim milk and though centrifuging reduced the amount, it may also have removed some other pro-oxidant. The experiments indicate the possibility of producing dry milk with excellent keeping qualities.

W.V.P.

## 15. The Gas-Packing and Storage of Milk Powder. C. H. LEA AND T.

MORAN, Low Temp. Res. Sta., Cambridge, England, and J. A. B. SMITH, Hannah Dairy Res. Inst., Ayr. Jour. Dairy Res., 13, No. 2: 162-215. 1943.

The authors present the following summary of their extensive work:

“(a) Full-cream powders stored in the presence of up to 0.01 ml. of oxygen per g. of powder kept very well at both normal and high temperatures. This figure corresponds to 1% oxygen (after completion of desorption) in the free-space gas of a can of spray-dried powder packed to a bulk density of 0.55 g./ml., or to 0.5% of oxygen in a can of roller powder packed to a bulk density of 0.35 g./ml. Tallowiness was never definitely detected under such conditions, and there seems to be little or no advantage to be gained, at least so far as palatability is concerned, by improving on this figure. An atmosphere containing not more than 0.01 ml. of oxygen per g. of powder can therefore be considered an ideal pack for milk powder.

“(b) Powders stored in the presence of 0.02 ml. of oxygen per g., *i.e.*, approximately 2% of oxygen in the free-space gas of a can of spray-dried full-cream powder, also kept well, in fact when storage was at 15° C. (59° F.) it was often impossible to distinguish between such powder and others stored in the presence of much smaller amounts of oxygen. At the high temperature at 37° C. (98.6° F.) the powder was usually, but not invariably distinguishable from powders stored in lower concentrations of oxygen, but the difference was slight. Below 0.02 ml. of oxygen per g. of powder may therefore be considered a good commercial pack.

“(c) Powder stored in the presence of 0.03 ml. of oxygen per g., *i.e.*, approximately 3% of oxygen in the free-space gas of a can of spray-dried full-cream powder, kept quite well. The testing panel could usually distinguish between powder stored for long periods at 15° C. (59° F.) in this and in lower concentrations of oxygen, but the difference was still small and probably not important from a practical point of view. In view of the difficulty of gas-packing, spray-dried powders in very low concentrations of oxygen, values up to 0.03 ml. of oxygen per g. of powder can be accepted as satisfactory for a commercial pack. It should be possible to store full-cream powder under those conditions for several years without serious deterioration. A rather lower concentration of oxygen than 0.03 ml./g. might, however, be desirable if storage is to include exposure to high atmospheric temperatures.

“(d) Powder stored in the presence of 0.04 ml. of oxygen per g., *i.e.*, approximately 4% oxygen in the free-space of a can of spray-dried full-cream powder, developed a suspicion of ‘off’ flavor (mark 1.0) in about 7 months and a definite slight ‘tallowy’ odor and flavor (‘off’ flavor mark 1.5–2.0) in about 12 months at 15° C. (59° F.), but was little, if any, worse after 2 or 3 years’ storage. Though always considered usable and sometimes quite good, such powder was definitely inferior to samples stored in lower concentrations of oxygen, and so high a figure as 0.04 ml./g. should not be accepted as satisfactory. At 37° C. (98.6° F.) results were more unfavorable than at 15° C. (59° F.) and a sample of spray full-cream powder stored at this temperature was considered to have become unusable for part of the storage period, between 4 and 12 months, when ‘off’ flavor marks between 2.0 and 2.8 were recorded. The powder subsequently improved slightly to mark 2.0 at 12 and 16 months.”

S.T.C.

## DISEASE

### 16. Variations in the Occurrence of Bloat in the Steer Progeny of Beef

Bulls. B. KNAPP, JR., A. L. BAKER AND R. W. PHILLIPS, U. S. D. A., Beltsville, Md. Jour. Anim. Sci., 2, No. 3: 221–225. Aug., 1943.

The progeny of 13 registered Hereford bulls were used over a two-year period in this study. A highly significant difference existed between progeny groups in both years on the frequency of bloat. The results indicate that there are inherent differences between progeny of different bulls in ability to handle large quantities of feed without digestive disturbances and indicate the possibility of improvement in this characteristic by selection on the basis of progeny tests.

C.F.H.

## FEEDS AND FEEDING

### 17. Determinations of Metabolizable Energy of Feeding Stuffs for

Cattle. E. B. FORBES AND E. J. THACKER, Dept. Anim. Nutr.,

Pennsylvania State Col., State College, Pa. Jour. Anim. Sci., 2, No. 3: 226-230. Aug., 1943.

In working out a method for calculating metabolizable energy from the digestible nutrients, a comparison was made between experimentally determined metabolizable energy of a group of feeding stuffs and computed values according to the formula of (1) Bratzler, (2) Kriss and (3) Axelsson. Metabolizable energy values calculated according to the method of Axelsson were the most satisfactory for feeds other than silage. When the factor 3.3 was used in computing metabolizable energy of the digestible ether extract of silage along with Axelsson's factors for digestible protein and carbohydrates of roughages, total metabolizable energy, values of silages were in approximate agreement with values based entirely on experimental observations. C.F.H.

18. Die Eignung von Kartoffeleiweisspülpe als Milchviehkraftfutter. J. SCHMIDT AND J. KLIESCH, Univ. of Berlin. Züchtungskunde, 18, No. 2: 43-49. Feb., 1943.

Two groups of six cows each were fed 3 kg. per day of this protein pulp to replace an oil cake mixture. The results were unfavorable. This was repeated in another three-week experimental period. When the experiment was again repeated but with only 2 kg. per day, practically no difference between the two groups was obtained. It is tentatively concluded that this feed may be fed with good results if the rate is not in excess of 2 kg. per day. J.L.L.

19. Untersuchungen über die Verwertung von Harnstoffstickstoff durch wachsende Kälber. J. SCHMIDT AND J. KLIESCH, Univ. of Berlin. Züchtungskunde, 18, No. 1: 1-11. Jan., 1943.

Whether the nitrogen in urea could be used by growing calves to replace part of their protein needs was studied on two pairs of identical twin calves and in another experiment with three lots of five calves each. The first pair of twins was fed for 728 days. The data are presented for them in five consecutive periods. The second pair was fed for 436 days and the results are presented separately for four periods of this time. The three groups were fed over a period of 252 days. The basic ration was clover hay and dried beet pulp. The control groups received in addition a concentrate mixture thought adequate to cover their protein needs. The amounts of digestible crude protein ingested per day were the same, but the digestible true protein was 390 g. per day for the control group and only 190 g. per day for the amide group. The third group was given only as much digestible true protein as the amide group and barely over half as much digestible crude protein. The growth curves of the control group were far above those



of the other two. The growth curve of the group receiving the amide nitrogen was only a little above that of the group which received the same amount of true protein but no extra amide nitrogen. The authors conclude that both sets of experiments show that growing calves are not able to use the nitrogen of urea to any practical extent for growth. This agrees with their earlier and more extensive experiments on sheep. J.L.L.

## FOOD VALUE OF DAIRY PRODUCTS

20. **Reviews of the Progress of Dairy Science. Section D. The Nutritional Value of Milk and Milk Products.** S. K. KON, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. *Jour. Dairy Res.*, 13, No. 2: 216-241. 1943.

This is an excellent review of the literature on the nutritional value of milk and milk products covering the period from the beginning of 1940 to the end of 1942. 430 references. S.T.C.

## ICE CREAM

21. **Dried Whole Egg Powder. V. Definition and Properties of Low Grade Powders.** M. W. THISTLE, MARGARET REID AND N. E. GIBBONS, Natl. Res. Council of Canada, Ottawa. *Canad. Jour. Res.*, D, 21, No. 8: 267. 1943.

The point at which 50% of a panel of tasters regarded dried egg powder as unsuitable for human consumption coincided with a rating of 2.7 on a scale ranging from 10 for excellent, fresh egg, to 0 for repulsive material. The protein fraction of these low grade samples had deteriorated badly, as shown by fluorescence measurements. The fat fraction showed no evidence of peroxide oxygen formation however. O.R.I.

22. **Better Ices and Sherbets.** C. D. DAHLE, Pennsylvania State Col., State College, Pa. *Ice Cream Trade Jour.*, 39, No. 10: 30. Oct., 1943.

The author outlines the requirements for, and characteristics of, high quality sherbets and ices, pointing out the difficulties encountered in manufacture under present conditions. Particularly stressed are the necessity for controlling the overrun under 50%, preferably at about 35%; the use of corn sugars high in dextrin and such cereal products as oat and wheat flour for improving body and texture; the proper amount of sweetening from the standpoint of body and texture as well as flavor; and the necessity of considering the sugar content of fruit products used as flavors. Defects of ices and sherbets are also briefly considered. F.J.D.

23. **Wartime Trends in the Retail Ice Cream Store.** CHARLES PAINO, Natl. Assoc. Retail Ice Cream Mfgs. *Ice Cream Trade Jour.*, 39, No. 10: 26. Oct., 1943.

Results of a survey conducted among 80 companies representing 300 retail ice cream stores revealed that sugar and labor are the most important problems facing operators of stores. Only 2% of the stores have discontinued the sale of cones and in these cases only intermittently. Eighty per cent have discontinued the sale of bulk carry-out ice cream and the other 20% have done so at times, but only 2% have discontinued the sale of packages. Sales of sherbet have ranged from 10% to 50% of the total gallonage with the big majority reporting about 25%. Over half the stores report that the consumer does not like to be forced to buy sherbet with ice cream. All of the stores are confident that they will be able to continue in business during the war.

F.J.D.

24. **Possible Conversion of Ice Cream Plants to Frozen Foods.** F. L. THOMSEN AND RICHARD GABEL, *Bur. Agr. Economics, U. S. D. A.*, Washington, D. C. *Ice Cream Trade Jour.*, 39, No. 10: 38. Oct., 1943.

This article is the first report of a survey being conducted with the object of determining the practicability of utilizing the resources of the ice cream industry for the freezing and distribution of frozen fruits, vegetables, meats and fish should future conditions in the canning industry demand it. The possibilities of a shortage of tin, should the war with Japan be long drawn out, are pointed out as are also the lack of refrigerator cars and the fact that greater utilization of fresh products is difficult. Some arguments for conversion of a small proportion of the ice cream industries' facilities at an early date rather than a substantial conversion at a later date are advanced. The general theme of the article seems to be that it would be better to play safe by conserving the available tin supply in some such manner rather than to hope for the best and be left high and dry with no tin for even the essential uses.

F.J.D.

## MILK

25. **Dipper Strainer—Flaky Milk.** H. J. BRUECKNER, *Cornell Univ.*, Ithaca, N. Y. *Amer. Milk Rev.*, 5, No. 1: 10. 1943.

The dipper-strainer, with fine mesh screen bottom, is being used rather successfully at milk stations for detection of mastitis milk. Flakes of fat may be distinguished from curd flakes, and are caused by improper handling of milk at the farm, chiefly by air cooling or slow cooling, mixing warm with cooled milk, and cooling milk in tanks not sufficiently filled with water to

reach the surface of the milk in the can. This method for examination of milk gives promise of much wider adoption. P.S.L.

26. Prospects for Milk Supplies in 1944. T. G. STITTS, Food Distrib. Admin., Washington, D. C. Ice Cream Trade Jour., 39, No. 10: 22. Oct., 1943.

Indications are that milk production in 1943 will be slightly less than in 1942 and estimations for 1944 are less than for 1943. Serious shortages of butter, cheese and fluid milk have developed in many areas. These are attributed to increase in the consumption of fluid milk and the greater utilization of dairy products by the armed forces and for Lease-Lend purposes. Recent Order No. 79 aims to prevent further increase in the consumption of fluid milk in order that supplies will be available for butter, cheese, and concentrated products. Notwithstanding Order No. 8 which reduced the milk solids going into ice cream by 35%, the total gallonage of frozen dairy foods for 1943 will be no less than in 1942, although it is estimated that the equivalent of 100,000,000 pounds of butter and 60,000,000 pounds of dry skim milk will have been saved.

The outlook for 1944 is that milk and dairy products will fall short of supplying demands. Subsidies of 25 to 50 cents per cwt. of milk to farmers and 3 to 6 cents per pound of fat for cream have been authorized in an effort to stimulate production.

No immediate change in the quota of milk solids for ice cream purposes is contemplated, but should the demand for milk solids for other, more urgently needed, dairy products become increasingly unsatisfied a review of existing food orders affecting dairy products would become necessary.

F.J.D.



# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

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New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### BOOK REVIEW

27. **Drying and Dehydration of Foods.** HARRY W. VON LOESECKE, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture. Published by Reinhold Publishing Corporation, 330 West Forty-second Street, New York. Consists of 10 chapters, glossary of terms, patent list, index. 302 pages. \$4.25.

The tremendous wartime use of dehydration as a means of enabling efficient preservation and movement of foods in the light of packaging and transportation problems has led to many developments in the art. The dairy industry has experienced a phenomenal increase in dehydration in its own field. A knowledge of the processes and developments in other food product fields is important to workers in the dairy industry because of a potential utilization of milk in dehydrated processed foods. The book, *Dehydration of Foods*, is written in an excellent review manner, devoid of quotations and citations. The references necessary to the subject are listed separately at the end of each chapter. Types of dehydrators includes illustrations of the principal types of units employed in the dehydration industry. A short resume is included in this chapter on the air, heat, moisture relationships of a tunnel dryer. Other chapters are: Sun drying and dehydration of fruits; Dehydration of Vegetables; Dehydration of Eggs; Milk and Butter; Dehydration of Meat, Fish, and Beef Blood; Plant Sanitation; Costs of Dehydration; Nutritive Value of Dried and Dehydrated Foods; Packaging and Storage; Methods of Analysis, and Reconstitution of Dehydrated Foods. The section on dehydration of milk contains no information not available in recognized dairy texts. No discussion is included of specific techniques and problems of roller drying of skim and whole milk, nor of the quality standards for dried milk. The material in this chapter is brief in the light of the importance of the industry. The discussion in the text is well organized, and easily readable, and enables a ready grasp of principal methods in food dehydration. It will be an excellent book for those in the dairy industry seeking a good review of other branches of the food dehydration industry, and their methods.

K.G.W.

### BREEDING

28. **An Analysis of Milking Shorthorn Records.** W. L. GAINES. Ill. Agr. Expt. Sta. Bul. 498. Aug., 1943.

Because milk records for Milking Shorthorn cows had never been analyzed in the same way as those for other breeds, data from Volumes 9 to 23 (1924-1938) of the Milking Shorthorn Year Book were collected and studied. The records were studied as a whole and in groups according to

number of milkings daily, length of calving interval, and length of record. Only records which included age of cow at calving, length of record, milk-fat yield, and fat percentage in addition to milk yield were studied. Milk-energy yield was computed by the usual formula. For the 6,311 records the average yield was 8,337 pounds of milk, 330 pounds of fat, and 8,285 pounds of FCM, and the average fat percentage was 3.97. The subgroups differed greatly in average milk yield, milk-fat yield, and FCM yield, but differed very little in average fat percentage. For the records as a whole, the correlation between fat percentage and milk yield was  $-0.217$ ; between fat percentage and fat yield,  $+0.106$ ; and between fat percentage and FCM yield,  $-0.026$  (not significant). Similar correlations were found in each of the subgroups. When the change in yield between 3.0% fat and 5.5% fat was expressed by a straight line, milk yield showed a decrease of about 30%, fat yield increase about 30%, and FCM showed very little change. These records afforded an opportunity to check the age-correction factors previously used for Milking Shorthorns, which were based on records of the breed up to June 1, 1920. The records reported here show a distinct shift toward earlier maturity, amounting to 6 months; and the age-correction factors need to be adjusted accordingly. There is no way of knowing whether this earlier maturity represents a change in the dairy qualities of the breed or a change in management of the cows. Actually age correction is probably simply an indirect allowance for live weight increases with age. A system of milk-yield correction based on live weight would be biologically more sound than an age-correction system, at least for cows less than 13 years old. The season in which a cow calved had an appreciable effect on FCM yield. In general August calvers had the lowest yield and November calvers had the highest. Certain of the records were studied to discover the difference between FCM yields of cows milked three times a day and of cows milked twice a day. The records of a typical group showed that cows milked three times a day exceeded in yield those milked twice a day by 39%. The standard for dairy cows of the Bureau of Dairy Industry is that cows milked three times a day should outyield those milked twice by 24%, so it appears that Milking Shorthorns respond well to the more favorable conditions associated with three milkings daily. A new table of age-correction factors for the Milking Shorthorns is given together with notes explaining its adaptation for use with other dairy breeds.

11 figures, 8 tables.

J.G.A.

## BUTTER

29. The 50-45-40 Method of Making Butter. G. H. WILSTER, Oreg. State Col., Corvallis. Natl. Butter and Cheese Jour., 34, No. 11: 16. Nov., 1943.

See Abstract No. 534, page A229, Vol. 26, No. 12, Dec., 1943.

W.V.P.



## CHEESE

30. What About the Tentative New U. S. Cheese Grades? WALTER V. PRICE, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., 34, No. 11: 8. Nov., 1943.

The tentative U. S. grades on cheddar cheese are unnecessarily complicated in defining grades for the primary market and unjustified in extending similar definitions and grade names to the consumer trade in cured cheese. These tentative grades should be carefully studied by graders, trade associations, consumer groups and commercial organizations and public hearings should be held by the Food Distribution Administration to review and, if necessary, revise these grades. W.V.P.

31. Milk Filtering in the Cheese Factor. RAYMOND MIERSCH AND WALTER V. PRICE, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., 34, No. 12: 26. Dec., 1943.

Filtration of milk at the cheese factory does not justify the acceptance of insanitary milk. The capacity of a given filter varies with the condition of the milk, such as its cleanliness, viscosity, acidity, fat and curd content. Preheating of the milk is sometimes used to increase filter capacity. Efficiency of a given filter is affected by the type of pad used, the re-use of washed pads and the volume and the sanitary condition of the milk. Clarification by centrifugal force is being used effectively in the Swiss cheese industry. Costs of filters vary from \$150 to \$600 for commonly used types and up to \$1300 for larger models; filter pads cost from 35 cents to about \$1.50 per day. Manufacturers who try to wash and re-use a filter pad take an unjustified risk. W.V.P.

## CHEMISTRY

32. Pectin as an Emulsifying Agent. Comparative Efficiencies of Pectin, Tragacanth, Karaya, and Acacia. HARRY LOTZKAR AND W. DAYTON MACLAY. Western Regional Res. Lab., U. S. Dept. Agr., Albany, Calif. Jour. Indus. and Engin. Chem., Indus. Ed., 35, No. 12: 1294. Dec., 1943.

The emulsifying ability of pectin is compared to that of gums tragacanth, karaya and acacia. The importation of these gums has been curtailed by shipping difficulties. The study was made of aqueous emulsions of olive, cottonseed and mineral oils under various conditions of acidity, ratio of oil to water and concentration of agent. Changes in the emulsions were followed by measuring the pH, viscosity, and specific interfacial area of the dispersed oil. In general the tragacanth stabilized emulsions were coarse and viscous, the acacia emulsions were fine and fluid, the karaya emulsions were gelatinous and the pectin emulsions were fine and viscous. The varia-

tion of emulsifying efficiency with acidity and from oil to oil limited generalizations that could be made on the comparative emulsifying efficiencies of pectin and the gums. B.H.W.

33. **Refractive Index Nomograph for Liquid Fatty Acids.** D. S. DAVIS, Wyandotte Chemicals Corp., Wyandotte, Mich. *Jour. Indus. and Engin. Chem., Indus. Ed.*, 35, No. 12: 1302. Dec., 1943.

Data in the literature covering the variation in refractive index with temperature, in the liquid state, for the normal saturated fatty acids from caproic to stearic, enabled the author to construct the accompanying chart for determining the refractive indices of these acids at any temperature in the range of applicability. B.H.W.

### CONCENTRATED AND DRY MILK; BY-PRODUCTS

34. **Dry Whole Milk—The Relation of Lecithin to Its Keeping Qualities.** C. D. DAHLE AND D. V. JOSEPHSON, Dairy Dept., Penn. State Col., State College, Pa. *Ice Cream Trade Jour.*, 39, No. 11: 22. Nov., 1943.

The stability of dry whole milk toward the development of oxidized flavor during storage was found to be improved by the partial removal of lecithin normally found in the fat globule adsorption layer. This removal was accomplished by separating the milk, churning the cream and rendering the butter. The resulting butter oil was remixed with the skimmilk before homogenizing, concentrating and drying on a small open roll drier. In one trial the fluid skimmilk was supercentrifuged before mixing with the butter oil. This apparently removed some of the lecithin found in the skimmilk and the dry product exhibited further improvement in keeping quality. Lecithin contents of the dry milks were 0.753, 0.480, and 0.366% (on the basis of the fatty extracts) respectively, for the control product, the butter oil and normal skimmilk product and the butter oil and supercentrifuged skimmilk product.

Improvement in keeping quality of dry whole milk resulting from a partial removal of lecithin was noted in both normal and nitrogen packed containers. F.J.D.

### DISEASE

35. **Septic Sore Throat Epidemic at School.** FRED W. CAUDILL, M.D., M.P.H. Dir., Div. of Communicable Diseases, AND MELVILLE A. MEYER, State Milk Sanit., Bur. of Foods, Drugs and Hotels, State Dept. of Health of Kentucky. *Jour. Milk Technol.*, 6, No. 4: 221. July-Aug., 1943.

A study is reported of two outbreaks of septic sore throat, which occurred among the students, teachers and other employees of the school.

Hemolytic streptococci were cultured from specimens of milk taken from the udders of 3 of the 10 cows in the herd furnishing milk for the school. All of the 10 cows showed the presence of *Staphylococcus aureus* or *albus* in the specimens of milk. The *Staph. aureus* were hemolytic in every instance.

It was thought that one of the boys, who helped with the milking and who reported to the infirmary with a sore throat, which appeared to be an extremely septic throat, was the source of the infection. A period of 7 days had elapsed between his reporting to the infirmary and the occurrence of the first large number of onsets. Improper cleansing and sterilization of milk utensils, milker's hands, cows' udders, and inadequate cooling were thought to be factors contributing to the outbreak.

L.H.B.

36. **Newer Methods for Control of Mastitis.** G. R. SPENCER, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., 34, No. 11: 48. Nov., 1943.

Find infected cows by regular use of strip cups and the Hotis test; segregate them, in separate barns if possible; and use separate teat cups on infected cows. Keep barns clean. Feed less grain to reduce production of infected cows. Milk cows regularly and thoroughly. Medicinal treatments for mastitis as yet are not wholly effective.

W.V.P.

37. **Some Results with Mastitis at Michigan State College.** R. C. HORWOOD, C. F. CLARK, AND C. S. BRYAN. Reprint from Mich. Agr. Expt. Sta. Quar. Bul., 26, No. 1: 43-50. Aug., 1943.

At the start of this program in 1932 there were 56 cows in milk in the college herd. Of these 53.5% had infectious mastitis. In November, 1941, the herd passed its first completely negative test. During the following 13 months to January 1, 1943, there have been nine months without any reacting cows. Acute systemic infection has increased and resulted in an increased loss from death or functional use of the udder. The results in lowering infection in the herd were obtained by culling the infected cows, preventing the spread of the organism, and by recovery by treatment of 16 mastitis-infected animals by lactovaccine and tyrothricin. Thirty-six non-infected cows produced 45% of the milk and 44% of the butterfat from the fore quarters. A reduction of 32% in milk and 32% in butterfat was found in a similar study of 9 cows with opposite-infected and non-infected rear quarters. A comparison of microscopic method, chloride determination, physical examination of udder, leucocyte determination, thymol test, physical examination of milk and culture of milk on blood agar plates, of testing for infectious mastitis on individual quarters, from November, 1932, to February, 1937, on the college herd led to the adoption of the monthly microscopic system of testing. There was no significant variation found in the milk nor udders of three mastitis-infected cows receiving 50

one-hour daily treatments with the short-wave diathermy as compared with three untreated mastitis-infected cows. Lactovaccine was found to correct 26% of the cows positive to streptococci infection. Fifty per cent of the animals responding did so from four treatments, others required up to 12. Treatments beyond that number gave no correction. The study did not indicate that an immunity to infection was developed in non-infected cows. Nine cows treated with tyrothricin became negative to infectious mastitis. A temporary effect which resulted in lower milk production and the production of abnormal milk was observed. Four animals that were treated within three weeks following infection all maintained or showed no increase in production in the lactation following treatment. Only one animal with long-standing infection responded in this manner. Three of the remaining four animals with long-standing infection (10 months to nine years) later developed acute systemic mastitis and died or lost the functional use of the udders. Three other cows in the herd not previously infected were lost during the same period. J.G.A.

## FEEDS AND FEEDING

38. Relationship between Fat Content of Dairy Grain Mixtures and Milk and Butterfat Production. C. F. MONROE AND W. E. KRAUSS. Ohio Agr. Expt. Sta. Bul. 644. 40 pages, illus. Aug., 1943.

Five feeding trials involving a total of 128 cows were conducted in order to determine the effect of different levels of fat in the grain mixtures on milk and butterfat production. The grain mixtures used contained only natural and by-product feeds commonly used in practice. The various fat levels were obtained, with some slight exceptions, by supplementing basal mixtures with either 41% (expeller) soybean oil meal, both with and without ground soybeans, or 44% (browned extracted) soybean oil meal. The continuous type of feeding trial was employed in all five of the trials summarized. With the exception of six Jerseys used in one trial in the second series, the cows were all Holsteins of fairly high milk-producing ability. In the first series of two trials involving 70 cows fed on three levels of fat, the average production of 4% (F.C.M.) milk per 30 days was: pounds, high-fat, 932.1; medium-fat, 921.1; and low-fat, 923.9. In the second series of three trials involving 58 cows fed the two levels of fat, the average production of 4% (F.C.M.) milk per 30 days was: pounds, high-fat, 1,077.5; low-fat, 1,054.3. The butterfat tests and liveweight gains were apparently not affected by the fat levels in the feed. There was also no noticeable difference in palatability in the grain mixtures used. There was no noticeable difference in their effect on the general health and condition of the cows or in preventing or causing udder trouble. Under the conditions of these trials, no significant differences were observed in the production of milk,

butterfat, or 4% (F.C.M.) milk or in the general health of milking cows from the feeding of practical grain mixtures ranging in average fat percentage from 4.89 to 2.69.

19 tables, 5 figures, 23 references.

J.G.A.

39. The Use of Dried Whey and Blood Meal in the Raising of Calves on Limited Amounts of Milk. I. L. HATHAWAY, G. W. TRIMBERGER, AND H. P. DAVIS. Neb. Agr. Expt. Sta. Res. Bul. 132. 19 pages. Oct., 1943.

Fifty grade Holstein heifer calves were successfully raised from approximately three weeks to six months of age on alfalfa hay, a grain mixture, a vitamin concentrate, and various amounts of skim milk, supplemented with a mixture composed of 3.2 parts of dried whey to one part of blood meal. Six and eight-tenths pounds of this mixture were used to replace 50 pounds of skim milk. The milk was fed at 50-, 100-, 150-, 200-, 250-, and 300-pound levels. The calves in five of the lots made an average daily gain of approximately  $1\frac{1}{2}$  pounds per head for 21 weeks. There was no statistically significant difference among the average gains in weight made by five of the six lots of calves. Three hundred pounds of skim milk alone produced cheaper gains than 50 pounds of skim milk supplemented with 34 pounds of the whey-blood-meal mixture. It was concluded that 6.8 pounds of a mixture composed of 3.2 parts of dried whey and one part of blood meal is a satisfactory substitute for 50 pounds of skim milk in the feeding of healthy dairy calves, which are approximately three weeks of age and which weigh not less than 104 pounds. Thirty-four pounds of this whey-blood-meal mixture can be fed, without serious effects, in 30 days even though as much as 1.8 pounds are fed daily for a few days. Healthy, vigorous dairy calves can be satisfactorily raised from three weeks to six months of age on as little as 50 pounds of the skim milk if it is properly supplemented with dried whey, blood meal, alfalfa hay, a grain mixture, and a vitamin concentrate. Labor can be saved in the raising of dairy calves by replacing the pail feeding of milk over a long period with a few weeks of milk feeding followed by a suitable grain mixture, alfalfa hay, and a vitamin concentrate. With the prices of feeds as quoted herein, the feed cost of raising calves on milk only may be less than when the milk is replaced by this whey-blood-meal mixture. However, when the labor cost is considered in connection with the feed cost, the additional expense of using the substitute mixture will not be prohibitive. Dried whey and blood meal can be utilized as a means of diverting milk from calf feeding to human food and to other uses.

J.G.A.

40. The Nutritive Value of Korean Lespedeza Proteins and the Determination of Biological Values of Proteins for Growing Dairy

**Heifers.** E. W. SWANSON AND H. A. HERMAN. Mo. Agr. Expt. Sta. Res. Bul. 372. 68 pages, illus. Aug., 1943.

An investigation was made of the utilization of the crude protein ( $N \times 6.25$ ) of Korean lespedeza hay and seed and various other feeds by growing dairy heifers. Biological values of the various proteins were determined, and net protein values and other measures of the nutritive value of the proteins were calculated. The net utilization of proteins from lespedeza hay, alfalfa hay, dried skimmilk, corn, lespedeza seed, soybean oil meal, and combinations of lespedeza hay with corn, milk, or soybean oil meal was not significantly different for dairy heifers when they were fed at a 10% level. The feeds were ranked according to the biological value of their proteins as follows: lespedeza hay, corn and lespedeza hay, milk and lespedeza hay, alfalfa hay, corn, soybean oil meal, soybean oil meal and lespedeza hay, lespedeza seed, and dried skimmilk. It was concluded that the quality of the absorbed proteins from Korean lespedeza hay or seed was equal to the quality of the absorbed proteins from milk, corn, alfalfa hay, or soybean oil meal for growing dairy heifers. The digestibility of the crude protein of lespedeza hay was shown to be relatively low, and the high lignin content of lespedeza leaves was revealed as a possible explanation of the poor protein digestibility. Highly lignified late-cut lespedeza hay was shown to be of very low nutritive value, the digestibility of all of its nutrients being greatly depressed. Digestion coefficients were determined for all of the nutrients of intermediate-cut lespedeza hay, late-cut lespedeza hay, and ground lespedeza seed.

12 figures, 16 tables, 110 references.

J.G.A.

## HERD MANAGEMENT

41. **Faster Milking.** W. E. PETERSEN. Univ. of Minn. Agr. Ext. Serv. Folder 119. Illus. June, 1943.

The technique of faster machine milking is briefly set forth with reasons for the several steps. Rules are as follows: (1) stimulate the cows to let down their milk one minute before putting on the machine; (2) operate the milking machine according to the manufacturer's directions; (3) strip the cows by machine; and (4) do not leave the machine on the cow after milk stops flowing.

J.G.A.

## ICE CREAM

42. **The Preparation and Use of Invert Syrup in the Manufacture of Ice Cream.** E. L. FOUTS, L. E. MULL AND T. R. FREEMAN, Univ. of Fla., Gainesville. Fla. Agr. Expt. Sta. Bul. 393. Sept., 1943.

Invert syrup of 70% solids was made using various acids and heating times. It was found that a satisfactory syrup could be made by boiling for

30 minutes 100 grams of tartaric acid, 100 pounds of sugar and 44 pounds of water. Citric acid in the same amount was found to be equally satisfactory. Three pints of lemon juice in the same formula produced equally satisfactory results and can be substituted for the tartaric and citric acids which are difficult to obtain. The invert syrup will keep well at room temperature.

In ice cream the invert syrup was found to be equal in sweetening value to the same amount of cane sugar and it is recommended that 25 to 50% substitutes of cane sugar can be made in ice cream, but where the latter amount is used the serum solids of the mix should be increased 1%.

C.D.D.

## MILK

43. Is It Desirable to Simplify and Unify Our Milk Quality Program. From the Viewpoint of the Health Officer. N. O. GUNDERSON, Commr. of Health, Rockford, Ill. Jour. Milk Technol., 6, No. 4: 225. July-Aug., 1943.

A four-point test system in use in Rockford, Illinois, is described. This system, after an initial inspection of the farm, shifts the point of observation with reference to quality control from the farm to the receiving dock or city plant. The four-point test used for judging the quality of milk is (1) Sediment test, (2) Direct microscopic (or Reduction test), (3) Phosphatase test, and (4) Swab test, together with other simple observations made at the receiving or processing plant. Under this system farm inspections are limited to the small number of non-compliance milk producers.

The cost of supervising this plan is approximately 0.155 cents per gallon of milk.

Preliminary results of this system of inspection at Rockford for the first five months of 1943 are given.

Some advantages given for this system are as follows:

1. A method of unifying milk control procedures.
2. Quickly locates apparent source of poor milk.
3. A means of culling out low-producing mastitis cows.
4. Minimizes time-consuming routine farm inspection.
5. Emphasizes safety, quality, and flavor of milk—not esthetic scoring of milk control.
6. Is apparently applicable to not only fluid milk, but to cream, butter, cheese, evaporated and dried milk quality control procedures.
7. Conserves manpower so essential in the war effort.

A suggestion is made that a National Allied Dairy Products Council be formed for the purpose of formulating specific recommendations that will simplify and unify the many varied milk control procedures applied to fluid milk, cheese, cream, butter, ice cream, evaporated and dried milk. L.H.B.

44. **Homogenized Milk and Public Health.** G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing, Mich. Jour. Milk Technol., 6, No. 4: 214. July-Aug., 1943.

The history and development of homogenized milk are given together with a very good bibliography on the subject.

Some of the facts regarding homogenized milk and particularly those having a public health significance are discussed. The factors considered are:

1. Homogenized milk must be a pasteurized product. Raw homogenized milk will develop a rancid flavor in a very short time.

2. Homogenized milk may be pasteurized at a higher temperature and maintained for a longer holding period than regular pasteurized milk. There is no cream line on homogenized milk, which will be destroyed by high pasteurization temperatures.

Homogenized milk cannot be mixed with raw milk. Rancid flavor will develop. Only clean milk may be used for homogenizing, otherwise, sedimentation is a possibility. Homogenization, when applied to milk, has a tendency to make the processor more "milk conscious" as it requires more care in processing and merchandising than does unhomogenized milk. Homogenized milk is a factor in developing increased milk consumption, due principally to the fact that homogenization protects against oxidized flavor.  
L.H.B.

45. **Milk House Construction, Equipment and Maintenance.** H. A. BEN-DIXEN, State College of Wash., Pullman, Wash. Jour. Milk Technol., 6, No. 3: 175. May-June, 1943.

Studies were made at State College on milk house construction and equipment suitable for Washington conditions.

Location, size and construction of milk houses are discussed together with types of materials best suited.  
L.H.B.

46. **Market Homogenized Milk in Philadelphia.** BERNARD SPUR, Milk Res. Labs., Children's Hosp. of Philadelphia, Philadelphia, Pa. Jour. Milk Technol., 6, No. 5: 266. Sept.-Oct., 1943.

That the sanitary quality of the homogenized milk produced in Philadelphia is exceptionally good is shown by the study made at the Milk Research Laboratories, Children's Hospital of Philadelphia, during the eight months period from December, 1940, to September, 1941. Samples were taken each month during this period, with the exception of January and March. The samples were purchased at the distributing platforms of the milk plants.

Of 34 plants producing Grade A homogenized milk, 26 of them had average counts under 8,000. This represented 96% of the total Grade A



homogenized milk consumed in Philadelphia. Only three plants had average counts over 25,000.

There were 36 plants producing Grade B homogenized milk, and of these plants, 22 had average bacterial counts under 8,000. This represented 89% of the Grade B homogenized milk consumed. All but two of the plants had average counts under 50,000 and only one was over 100,000: Twelve of the dairies were known to pasteurize before homogenizing, while three were known to homogenize before pasteurizing.

The bacterial counts obtained in these plants seemed to indicate that the question of getting low counts is more dependent on the sanitary conditions in the plant (and probably on the type of bacteria in the raw milk supply) than on the order of the processes. Of the twelve plants pasteurizing before homogenizing, only one had an average bacterial count over 5,000, and that was an average count of 18,200 on the Grade B milk. The average bacterial count on this plant's Grade A milk was 4,018. Of the three plants pasteurizing after homogenization, two of them had very low counts on both Grade A and B milks (less than 1,400). The other plant had average counts of 15,338 on the Grade A and 11,681 on the Grade B.

The average curd tension of the Grade A milks was 11.6 gms. and on the Grade B was 11.2 gms. The curd tension for the Grade A milks ranged from 5.3 gms. to 18.4 gms., while for the Grade B milks it ranged from 4.6 to 15.9 gms.

L.H.B.

47. **A Ropy Milk Outbreak Caused by a Thermoduric Micrococcus.** C. C. PROUTY, Div. of Dairy Husbandry, Wash. Agr. Expt. Sta., Pullman, Wash. Jour. Milk Technol., 6, No. 5: 263. Sept.-Oct., 1943.

An outbreak of ropy milk occurring in commercially pasteurized milk was studied. An organism closely related to *Micrococcus freudenreichii* was isolated and its source was traced to one of the dairies furnishing milk to the pasteurizing plant. When milk from this producer was excluded, no further ropiness was experienced in the pasteurized product.

Some cultures of the organism were found to survive a pasteurization temperature of 143° F. for 35 minutes, while in other instances 20 minutes at that temperature was sufficient to render the organism nonviable.

The organism had the ability to cause rapid development of ropiness with an abnormal flavor and odor, followed by active proteolysis. L.H.B.

48. **The Connecticut Three-Point Laboratory Program as an Aid to Control of Pasteurized Milk.** FRIEND LEE MICKLE AND EARLE K. BORMAN, Dir. and Asst. Dir., Bur. of Labs., Conn. State Dept. of Health, Hartford, Conn. Jour. Milk Technol., 6, No. 4: 231. July, Aug., 1943.

A three-point program for determining the quality of pasteurized milk

was started in 1937 after a state-wide survey. The tentative standards set were:

- "1. Direct microscopic clump count limits:  
Grade A pasteurized milk—200,000 per ml.  
Grade B pasteurized milk—500,000 per ml.
2. Coliform organisms: Absent in 0.1 ml.
3. Phosphatase test: Less than 0.05 mg. phenol."

The coliform standard was changed to *absent in 1.0 ml.* in January, 1941.

The use of these standards has not worked a hardship on dealers in good quality milk.

The direct microscopic clump count on pasteurized milk has proven satisfactory in easily classifying milk as either good or bad and with greater rapidity than the agar plate count. In 1939 only 4.1% of the Grade A samples had counts in the borderline range between 200,000 and 300,000, while only 6.5% of the Grade B samples had counts in the borderline range between 500,000 and 1,000,000.

During the five-year period 77.4% of the samples (more than 15,000) met the tentative standards of 200,000 for Grade A and 500,000 for Grade B pasteurized milk by the direct microscopic clump count; 75.6% met the tentative standard for coliform organisms; and 92.7% met the tentative standard for the phosphatase test.

The advantages of the three-point program for determining the quality of pasteurized milk were given as follows:

"1. The direct microscopic clump count presents a more nearly true picture of the bacteriological quality of a milk supply than does the plate count, and provides a much more rapid, more complete, and hence more effective laboratory service to the control official.

2. Tests for coliform organisms, furnishing evidence of improper handling after pasteurization, yield supplementary information correlating well with the direct count.

3. The phosphatase test yields the only positive information obtainable on the effectiveness of pasteurization, and hence is an indispensable index of probable safety of a supply."

L.H.B.

49. Efficiency of Milk Marketing in Connecticut. 5. Economics and Biology of Alternate-Day Milk Delivery. R. G. BRESSLER, JR., E. O. ANDERSON, D. A. CLARKE, JR., AND E. N. BILENKE. Conn. Agr. Expt. Sta. Bul. 247. 60 pages. May, 1943.

Alternate-day delivery of milk has proved to be an effective method of conserving resources. Retail milk truck mileage has been reduced an average of 44% in Connecticut markets. The mileage savings and increased deliveries per stop reduced route time by nearly one-third, which in turn made possibly increased loads and some consolidation of routes. The pro-

gram reduced requirements for gasoline and tires by approximately 40%. Man-hours were reduced 30%, but some of this reduction represented shorter hours per route so that the number of men freed for other employment was only 14% in 1942. Under present conditions annual savings in Connecticut total one million gallons of gasoline, forty-six thousand quarts of oil, and two thousand truck tires, and the elimination of nearly 200 routes.

Monetary savings have not been large in spite of these important reductions in men and materials. In 1941, retail delivery costs in city markets average 4.06 cents per quart. In the spring of 1942 alternate-day delivery costs were approximately 3.70 cents per quart, while a year later, they were 3.61 cents. These savings were offset at least in part by wartime increases in the other costs of milk distribution. If alternate-day deliveries are continued in the post-war period, and if weekly earnings of routemen return to the normal pre-war levels, total delivery costs would be approximately 2.74 cents, a savings of 1.32 cents per quart.

Successful application of alternate-day delivery is dependent on the keeping qualities of milk. Experiments indicate that satisfactory results are obtained where home refrigeration is adequate. P.H.T.

**50. Efficiency of Milk Marketing in Connecticut. 6. Truck Costs and Labor Requirements on Milk Delivery Routes. D. A. CLARKE, JR., AND R. G. BRESSLER, JR. Conn. Agr. Expt. Sta. Bul. 248. 39 pages. June, 1943.**

In general, costs fell into three categories: overhead costs, fixed operating costs per day, and variable operating costs per mile. Combining overhead and fixed operating costs, the daily costs of operating retail trucks average \$1.68 plus \$0.44 per mile. For wholesale trucks daily costs averaged \$2.46 plus \$0.045 per mile. These costs are based on daily delivery conditions and on cost rates representative of the period 1940-41. The costs of operating alternate-day delivery trucks averaged about \$7.51 per day plus \$0.045 per mile during the spring of 1943.

In greatly simplified form the time required to operate daily retail routes may be represented by 1.2 minutes per quart plus four minutes per mile, while for alternate-day delivery the time requirements are 0.8 minutes per quart plus four minutes per mile. On wholesale routes the time per quart is only 0.35 minutes. Under average conditions of route organization it is possible to handle about 35 quarts per hour on daily retail delivery routes, 60 quarts per hour on alternate-day retail routes and 125 quarts per hour on wholesale routes.

With daily delivery, the average retail driver earned about \$44 per week and labor costs averaged about \$0.026 per quart. With alternate-day delivery average weekly earnings were \$56 (commission basis) while labor costs averaged \$0.025 per quart. On salary and commission payments the

weekly earnings averaged \$46 and costs \$0.025 per quart. On wholesale routes the average cost per quart was between \$0.006 and \$0.007.

In 1941 truck costs averaged \$0.011 per quart, route labor costs \$0.026, miscellaneous costs \$0.004, and total delivery costs of \$0.041 per quart. In 1943 with alternate-day delivery, truck costs averaged \$0.007, route labor \$0.025, miscellaneous route costs \$0.004 and total retail delivery costs \$0.036 per quart. In the spring of 1943 wholesale delivery route truck costs average about \$0.003 per quart, labor costs \$0.006, miscellaneous costs \$0.004, and total delivery costs \$0.014 per quart. P.H.T.

51. Efficiency of Milk Marketing in Connecticut. 7. Milk Delivery in Rural Connecticut. ALAN MACLEOD AND C. J. MILLER. Conn. Agr. Expt. Sta. Bul. 249. 37 pages. July, 1943.

Opportunities exist for conserving scarce resources in the distribution of fluid milk in rural areas. A study of 12 areas in Connecticut has indicated the size of the savings that might be realized from complete adoption of alternate-day delivery and from the allocation of exclusive territories with deliveries made daily or on alternate days.

In the summer of 1942 about 36 per cent of the daily delivery mileage was being saved. This compares with a maximum estimated potential savings of 45 per cent if all producers were placed on an alternate-day basis.

Adoption of a system of exclusive territories would yield potential savings of 38 per cent with daily delivery or of 64 per cent with alternate-day delivery. The present system of alternate-day and daily delivery is saving almost 1.3 million miles yearly in rural Connecticut towns. These savings could be increased to 2.2 million miles by the adoption of exclusive territories in combination with alternate-day delivery. This would produce a savings of about 220,000 gallons of gasoline, 11,000 quarts of oil, and 500 delivery truck tires. The savings in manpower would probably be most important of all as the time released from delivery can be put to more productive uses.

P.H.T.

## MISCELLANEOUS

52. Post-War Problems of the Locker Industry. SLEETER BULL, Univ. of Ill., Urbana. Natl. Butter and Cheese Jour., 34, No. 11: 22. Nov., 1943.

The rapid growth of the locker industry has resulted in some troubles caused by poor plant design, improper insulation, under equipment, lack of information in management and operation, and consumer ignorance. The future promises more problems such as the development of household units for home freezing and storage and the possibility of freezing foods in transit at high altitudes. W.V.P.

53. **Pyrex Glass Tubing as a Substitute for Metal Milk Pipe in Dairy Plants.** G. J. HUCKER AND ROBERT E. THOMAS, N. Y. State Agr. Expt. Sta., Geneva, N. Y. Jour. Milk Technol., 6, No. 4: 197. July-Aug., 1943.

A study was made under commercial conditions in a plant handling approximately 45,000 pounds of milk daily.

The study revealed that pyrex glass tubing, beaded or flanged, can serve as a substitute for metal milk pipe in dairy plants.

It proved impractical under general operating conditions to dismantle beaded glass tubing daily for cleansing, the same as metal pipe. Some breakage and chipping was experienced when this was done.

It was found that pyrex tubing could be satisfactorily cleaned in an assembled position.

A bacteriological study of glass tubing cleaned and sterilized without disassembling gave results, from a sanitary standpoint, which were satisfactory and comparable to those obtained when metal pipes are dismantled daily and cleaned in the usual manner practical in the dairy industry at present.

The method used for daily cleaning of the glass tubing in the assembled position was as follows:

Rinse milk lines by circulating (1) cold water, (2) a 0.6% solution of an alkali cleanser containing 4.0% of a wetting agent at a temperature not less than 110° F. for at least 20 minutes, (3) clean water rinse at 110° F., (4) hot water rinse at about 190° F. for not less than 15 minutes. Just before use circulate a chlorine rinse solution of at least 100 ppm. strength through the entire milk processing system. Glass tubing and tube joints opened and examined at intervals of two to six weeks using this treatment were found to be in an excellent sanitary condition.

The optimum time interval for disassembling and examining tubing and joints was not determined. It was suggested, however, that the interval should not be greater than two weeks, until further data were secured.

L.H.B.

54. **Comparative Educational Background of Dairy Graduates, Sanitary Engineers and Veterinarians in Milk Control.** SIDNEY SHEPARD, Birmingham, Ala. Jour. Milk Technol., 6, No. 4: 235. July-Aug., 1943.

"Since World War I the field of dairy science as it relates to public health has slowly but surely drifted from the dairy college graduates into the hands of sanitary engineers (a division of civil engineering) and veterinarians. Today, only sanitary engineers may hold commissions as milk sanitarians with the United States Public Health Service, and only veterinarians are deemed qualified to exercise sanitary supervision over the

production and manufacture of all products of 'bovine origin' being used by our armed forces."

From a study made of the curricula of five dairy colleges, four veterinary colleges and five schools of sanitary engineering ("In each instance the colleges studied were those generally accepted as outstanding in their particular field."), it was obvious that the dairy colleges were doing a good job in educating men for a career in milk sanitation. In every instance they had courses in dairy manufacture, animal husbandry, bacteriology, dairy bacteriology, chemistry, dairy chemistry and agricultural economics.

Of the veterinary colleges studied only two offered any courses in the fundamentals of milk and its products and these were of a limited nature. More courses were offered in animal husbandry, but this was due to the fact that farm animals other than cows were studied.

None of the schools of sanitary engineering offered any courses of study in either dairy manufactures or animal husbandry.

Thus, it would seem that the dairy college graduate would be the logical choice for milk sanitation work.

From a questionnaire sent to cities of more than 100,000 population, it was ascertained that in 32 out of 79 of these cities answering the questionnaire, no dairy graduates were employed in milk sanitation.

The fact that dairy graduates are being discriminated against is probably due to the fact that the dairy colleges have been too prone to overlook the public health field, concentrating their interest in turning out men for the industry; also, many health officials have become oblivious to the virtues of the technically trained dairy college graduate when milk sanitarians are being sought.

"While many milk sanitarians are sanitary engineers who by practice and experience have become expert in this particular line of endeavor, the preeminence of the educational background of the dairy graduate cannot be denied—by this virtue above all, is he (the dairy college graduate) the logical candidate for milk sanitation and all it implies." L.H.B.

55. Properties of Detergent Solutions. Thermal pH Coefficients of Alkaline Solutions. LESTER E. KUENTZEL, JAMES W. HENSLEY, AND LESLIE R. BACON, Wyandotte Chemicals Corp., Wyandotte, Mich. Jour. Indus. and Engin. Chem., Indus. Ed., 35, No. 12: 1286. Dec., 1943.

This is the fourth of a series of papers concerned with the properties of detergent solutions, especially those used in laundry practice. Detailed pH data at 25°, 40°, and 60° C. are presented for distilled water solutions of nine commercial alkalies sometimes used alone and in combination as soap builders. The alkalies examined were sodium hydroxide, sodium carbonate, sodium bicarbonate, trisodium phosphate, tetrasodium pyrophosphate,

sodium tetrphosphate, sodium metasilicate, sodium sesquisilicate and sodium orthosilicate.

B.H.W.

56. **Mixed Calcium Salts of Soaps and Anionic Detergents.** GILBERT D. MILES AND JOHN ROSS, Colgate-Palmolive-Peet Co., Jersey City, N. J. Jour. Indus. and Engin. Chem., Indus. Ed., 35, No. 12: 1298. Dec., 1943.

Mixed salts of calcium with fatty acids and synthetic anionic detergents were found to form when mixed in solution. Study of formation of the salts in mixtures containing sulfated detergents, soap and calcium salt permitted appraisal of decrease in foaming and deterative properties of the solutions. No corresponding behavior was found for magnesium salts.

B.H.W.

57. **Labor Saving Through Farm Job Analysis. I. Dairy Barn Chores.** R. M. CARTER. Vt. Agr. Expt. Sta. Bul. 503. 66 pages, illus. June, 1943.

A detailed record was made of the time taken, the distance walked, and the routes traveled by the owner in doing the barn chores for his 22-cow dairy. After careful study of the problem a series of changes, designed to make the work easier and to save time, were made. These changes were of four general types: (1) Rearrangement of the stable; (2) Improvement of work routines; (3) Provisions of adequate and suitable equipment; (4) Convenient location of tools and supplies. As a result, the time spent on chores was reduced from 5 hours 44 minutes to 3 hours and 39 minutes daily, a saving of 2 hours 5 minutes, and the travel was reduced from  $3\frac{1}{4}$  to  $1\frac{1}{4}$  miles daily, a saving of 2 miles. Two hours a day is equivalent to more than 60 12-hour days, a good 2 months work, in a year; 2 miles daily is equivalent to 730 miles yearly. The money cost of the changes made was small. What this man did can be done by any dairy farmer who will undertake the task seriously. Many of the ideas worked out on his farm can be applied on other farms without change. In other cases some modification may be necessary to make them workable. In still others new schemes may need to be devised to solve particular problems. But the method here used of observing the chores, studying the problem, and working out improvements can be applied anywhere. Specific suggestions for the job are given; also a score card for dairy barn layout.

38 figures, 22 tables.

J.G.A.





# JOURNAL OF DAIRY SCIENCE

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National Institute for Research in Dairying, Reading, England	The Royal Technical College, Copenhagen, Denmark
New York Association of Dairy and Milk Inspectors	United States Department of Agriculture

## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

58. Significance of Adequate Controls in Absolute Sterility Determinations. D. C. FOORD, C. L. CRANE, AND B. S. CLARK, Res. Dept., Amer. Can Co., San Francisco, Calif. Food Res., 8, No. 6: 489. Nov.-Dec., 1943.

Data accumulated in routine bacteriological examination of paper milk containers over a period of three and one-half years (30,718 tests) by the rinse test of the A.P.H.A. Standard Methods, indicated 78.5% were sterile. If a correction for technique error is made, 97% of the containers were sterile. The non-sterile containers showed fewer than three bacteria per container or approximately 0.003 of the limit permitted by the U. S. Public Health Service Milk Ordinance and Code.

Data are included which demonstrate that the method of incubating nutrient broth in the container, as suggested in Standard Methods, yields an inaccurate measure of the organisms which the container would contribute to milk placed therein, because the container is subjected to conditions which it is not required to meet in commercial use.

Control data for the rinse test indicate that in testing for absolute sterility of any type of containers no less than one control should be made for each three containers tested.

F.J.D.

59. Extracts from Irish Moss as a Substitute for Agar in Bacteriological Culture Media. A. W. WALKER AND A. A. DAY, Dept. of Bact., Northwestern Univ., Medical School, Chicago. Food Res., 8, No. 6: 435. Nov.-Dec., 1943.

The possibility of using the gelatinous extract of *Chondrus crispus* (Irish Moss) as a substitute for agar, which is becoming a "critical material," was investigated. The particular product used was "carrageen" made by the Krim-Ko Co. of Chicago, Illinois, to which the manufacturers have given the name "Carragar" when used as a substitute for agar.

Results indicated that "Carragar" can be used as a solidifying agent in bacteriological culture media. The gel is not as firm as agar, the melting point is lower and it is more easily hydrolyzed. However, it is satisfactory for most purposes and can be made firmer by additions of agar to it. Neutralization of the "Carragar" before heating prevents its hydrolyzation.

"Carragar" produced comparable results, in all of the media employed in the study, to those obtained with agar.

F.J.D.

60. A Comparative Study of One Per Cent and Five Per Cent Solutions of 30 to 40 Mesh Gelatins for Bacteriological Examination. TECH. COM. OF THE EDIBLE GELATIN MFGS., Res. Soc. of America. Food Res., 8, No. 6: 429. Nov.-Dec., 1943.

One per cent solutions of 30- to 40-mesh gelatin are preferable to five per cent solutions for bacteriological examination, in the judgment of the committee. While no results were obtained with coarsely ground or flake gelatins, it is suggested that five or ten per cent solutions will probably yield more reliable results. F.J.D.

61. Nutritional Studies on *Streptococcus Lactis*. I. An Unidentified Growth Factor Found in Yeast Extract. F. R. SMITH, Univ. of Calif., Davis. Jour. Bact., 46, No. 4: 369. Oct., 1943.

The author describes the preparation and some characteristics of an unknown substance in yeast extract that is essential for the growth of certain strains of *Streptococcus lactis*. This growth factor is apparently not a known vitamin and could not be replaced by a combination of amino acids. D.P.G.

62. Heat Resistant Organisms in Milk Supplies. W. D. DOTTERER, Dir. of Labs., Bowman Dairy Co., Chicago, Ill. Jour. Milk Technol., 6, No. 5: 269. Sept.-Oct., 1943.

It is quite common to find a great many milk supplies containing thermoduric bacteria. Some areas are a greater source of trouble from these organisms than others.

Utensils have generally been the source, with milking machines involved more frequently than others; however, the cow's udder may also be the source.

High-temperature short-time pasteurization will generally give higher counts than the vat system of pasteurization.

Great care in the cleansing and sterilizing of utensils is necessary for the elimination of thermoduric organisms from milk supplies. L.H.B.

## BREEDING

63. Inhibition of Sperm Glycolysis and Reversibility of the Effects of Metabolic Inhibitors. HENRY A. LARDY AND PAUL H. PHILLIPS, Dept. Biochem., Univ. Wis. Jour. Biol. Chem., 148, No. 2: 343. 1943.

Using a 0.02 M glucose solution as a substrate for washed bull spermatozoa the following inhibited glycolysis and their effect was reversible: cyanide, quinone, fluoride and azide; while maleate, hydroquinone, iodo-

acetate and glyceraldehyde inhibited glycolysis but their effect was not reversible.

“In yolk-buffer some specimens of bull spermatazoa could be almost completely inactivated by fluoride for several days and upon transfer to fresh yolk-buffer vigorous motility was regained.” A.O.C.

64. Inhibition of Sperm Respiration and Reversibility of the Effects of Metabolic Inhibitors. HENRY A. LARDY AND PAUL H. PHILLIPS, Dept. Biochem., Univ. Wis. Jour. Biol. Chem., 148, No. 2: 333. 1943.

The effect of 17 different metabolic inhibitors on the respiration of bull spermatazoa and the reversibility of the effect of these inhibitors is given. Cyanide, malonate and benzoate inhibit the respiration but not the glycolysis of the sperm and are toxic to motility only when glucose is absent. This is further evidence of the ability of 2 separate metabolic processes, *i.e.*, oxidative and glycolytic, to furnish energy for motility of bull sperm. A.O.C.

## BUTTER

65. There Will Always Be Quality. R. E. ELDRED, The Great Atlantic & Pacific Tea Co., Chicago. Natl. Butter and Cheese Jour., 35, No. 1: 8. Jan., 1944.

The customer today wants quantity rather than quality. Inferior cream and butter command the same prices as better products. Some factors causing quality deterioration are: delays in cream pick-ups, farm labor shortage, relative prices for farm and dairy products, curtailment of educational field work, discrepancies in official gradings, lack of food regulatory personnel, increased production of unsalted butter and differences of point values in farm and creamery butter. But quality has not been forgotten because critical customers are asking for better butter and the army, which is buying good table butter, is educating the tastes of more men. As normal trading conditions are resumed receivers will be more strict, the Food and Drug departments will again do routine checking and the natural pride of butter makers will all tend to improve quality. Large milk drying operations will produce fine cream, some of which will be used for butter, and small operators may have to develop cooperative field services to compete with these larger concentrators. W.V.P.

66. Looking into the Future of Creamery Operations. H. E. BEHLMER, Cherry-Burrell Corp., Chicago. Natl. Butter and Cheese Jour., 34, No. 12: 18. Dec., 1943; and 35, No. 1: 36. Jan., 1944.

Future efforts to improve quality and lower manufacturing costs will probably require changes in methods and machinery. Cream quality will

be improved by education of producers, development of better-equipped cream receiving units, and more efficient utilization of milk-solids-not-fat. Innovations in creameries will include: continuous butter manufacture without churns, more general use of the vacuum-type pasteurizer, better cooling practices, and coolers designed to control temperatures and deliver cream to churns instead of holding tanks. Other significant changes expected are: greater use of stainless steel in vats, pipes, pumps and refrigerating units, better sanitation, more general use of storage tanks instead of coil vats and the use of better lubricants. W.V.P.

**67. Determination and Content of Carotene and Vitamin A in Wisconsin Butter.** S. BERL AND W. H. PETERSEN, Dept. Biochem., Univ. Wis., Madison. *Jour. Nutr.*, 26, No. 5: 527-538. Nov., 1943.

A solvent extraction is described for the determination of carotene and vitamin A in butters, utilizing the yellow color of the carotene for its determination and the Carr-Price reaction for the determination of vitamin A.

Diacetone alcohol, 94%, proved to be superior to 92% methanol for the extraction of non-carotene pigments.

Twenty-two samples of butter made during the week of March 23, 1942, by creameries in southwestern Wisconsin, and 20 samples of butter from the same creameries in July, 1942; and samples of butter made in September and January collected from various sections of Wisconsin were used in this study.

The March butters averaged 9,500 I.U.; the July and September butters 18,000 I.U.; and the January butters 10,500 I.U. per pound. In the summer butters about 75% of the total natural butter pigment was found to be carotene, in the winter butters 60 to 65% of pigment was carotene.

Storage of butter at -22 to -23° F. as long as 8 months did not result in a loss of carotene or of vitamin A. C.F.H.

**68. The Iron Content of Butter and Its Relation to the Butter Wash Water.** J. B. LINNEBOE, Alberta Dept. Agr., Edmonton. *Sci. Agr.*, 24, No. 2: 64. 1943.

Samples of butter from 146 churnings representing 27 creameries were analyzed for iron. The iron content of the wash water from 26 of these creameries were also determined.

The iron content of the butter ranged from 0.4 ppm. to 3.3 ppm. The butter possessing the lower iron content tended to grade higher and there was also a tendency towards uniformity in the iron content of the butter from the same creamery, particularly the butter of first grade.

The iron content of the wash water was shown to affect the iron content of the butter. Where portions of churnings were removed from the churn and washed with distilled water or natural water, low in iron, all the first grade butter contained less than 1.0 ppm. of iron. O.R.I.

## CHEESE

69. Cottage Cheese from Soybean Curd? ANONYMOUS. Milk Dealer, 33, No. 3: 27-56. Dec., 1943.

Following a description of making cottage cheese from soybeans' milk, as released by Science Service, a survey of the possibilities of making a substitute cottage cheese by dairy plants is made. Methods of treating soybean milk to produce a curd comparable in many respects to regular cottage cheese made from skimmilk have been to culture milk with lacto-bacillus or soy acidophilus organism; to use an alkali (magnesium chloride) to coagulate the soybean milk or to curdle the milk with a weak acid. A patent for manufacturing has been applied for by one dairy plant. The possibility of providing a substitute cottage cheese under existing shortages of skimmilk, which possesses some of the texture, flavor, acidity and moisture content characteristics of regular cottage cheese is discussed. C.S.T.

70. Starters for Cheese Making. G. M. MOIR, Dairy Chemist. New Zeal. Dept. Agr. Bul. No. 162.

This bulletin brings together the more important research and practical observations on the making of starters as applied to cheese factory conditions in New Zealand.

Starters are used to ripen the milk before renneting, to secure proper acidity during the making process, to restrain undesirable bacteria, and to aid in cheese ripening. The starter must contain the right bacteria, and no others, in active condition capable of developing acidity at the proper rate. The cultures in New Zealand are often pure strains of *Str. cremoris*, but usually *Str. lactis* and *Str. citrovorus* are also present.

Bacteriophage is a common cause of limited acid development. The phage infection usually occurs from the "whey frog" from a whey separator, from dust, or from contamination from phage-infected starters. Some other conditions that cause slow acid development are low temperatures of incubation, growth-retarding substances in the milk, aeration of the milk after pasteurization, and too small inoculation, but phage contamination is most important.

The objection to excessive acidity (1.0-1.2%) in former years was due to poor starters. Actually 0.65-0.85% is quite satisfactory and overripening is no longer so common due to use of proper bacteria.

Special milk should be selected for starters. Special starter rooms in cheese factories are desirable. Several starters are preferred to one to assure against complete failure. Mother cultures are best carried in glass containers. Stress is given to cotton plugging the openings for inoculation for the main and mother starter and of flaming these openings when transfers are made to avoid phage contamination. Details of making the starters are given and the Whitehead and Cox vitality test is described.

It is pointed out that phages have not been troublesome in America and that mixed cultures are probably preferable to single strains to control phage difficulties.

A.C.D.

## CHEMISTRY

71. **Modifications of the Swift Stability Test.** R. W. RIEMENSCHNEIDER, J. TURER, AND R. M. SPECK, Eastern Regional Res. Lab., U.S.D.A., Philadelphia, Pa. *Oil and Soap*, 20, No. 9: 169-171. Sept., 1943.

An all-glass aeration tube for use in the accelerated method of determining stability of fats was found to have several advantages over the rubber-stoppered test tubes. An improved air-distributing apparatus is also described. The capacity of the apparatus has been increased three-fold by the adoption of a procedure which permitted the use of only one tube for each test sample.

Factors influencing the peroxide values as determined by adaptation of Wheeler's method were investigated.

J.L.H.

72. **The Development of a Practical Antioxidant for Lard and Shortening.** A. LIPS AND W. D. MCFARLANE, Dept. of Chem., Macdonald College (McGill Univ.), Ste. Anne de Bellevue, Quebec, Canada. *Oil and Soap*, 20, No. 10: 193-196. Oct., 1943.

Wheat germ oil, extracted by means of ethylene dichloride and fortified with citric acid, was found to be an efficient antioxidant in lard and shortening. The wheat germ oil supplied the phenolic type of antioxidant in the form of tocopherols and the citric acid, the acidic component necessary in an antioxidant intended for use in both animal and vegetable fats and oils.

J.L.H.

73. **The Solubility of Gases in Butter Oil, Cottonseed Oil, and Lard.** P. S. SCHAEFFER AND H. S. HALLER, Bur. of Dairy Indus., U. S. D. A., Washington, D. C. *Oil and Soap*, 20, No. 8: 161-162. Aug., 1943.

The solubility of hydrogen, oxygen, air, nitrogen, and carbon dioxide at 40° C. is slightly higher in butter oil than in cottonseed oil and lard. The solubilities of the above gases in butter oil at 40° C. and 60° C. are shown in the following table:

Gas	Gas dissolved in 100 ml. butter oil	
	40° C.	60° C.
	<i>ml.</i>	<i>ml.</i>
Oxygen .....	14.2	12.7
Nitrogen .....	8.9	7.9
Hydrogen .....	5.4	6.8
Air .....	10.1	9.6
Carbon dioxide .....	109.5	91.0

J.L.H.



74. The Application of the Ferric Thiocyanate Method to the Determination of Incipient Rancidity in Fats and Oils. A. LIPS, R. A. CHAPMAN, AND W. D. McFARLANE, Dept. of Chem., Macdonald College (McGill Univ.), Ste. Anne de Bellevue, Quebec, Canada. *Oil and Soap*, 20, No. 11: 240-241. Nov., 1943.

A colorimetric method for determining fat-peroxides in whole milk powder, previously reported by the authors, has been modified for use in fats and oils. The method is based on the oxidation of ferrous to ferric iron by the peroxides present in oxidized fats. The ferric iron is determined as ferric thiocyanate; the intensity of the color being measured with a Coleman spectrophotometer set at 485 m $\mu$ . J.L.H.

75. A Rapid Test for Alpha Dicarboxyls. L. O'DANIEL AND L. B. PARSONS, Res. Dept., Lever Bros. Co., Cambridge, Mass. *Oil and Soap*, 20, No. 4: 72-74. Apr., 1943.

When alcoholic caustic potash is added to auto-oxidized fats and oils more or less highly-colored solutions are formed, the color depending upon the type of fat and the extent of oxidation. The color is probably due to quinoid compounds formed by aldol condensation of alpha-diketones in a manner analogous to the formation of para-xyloquinone from diacetyl. Linoleic and possibly linolenic acids or esters are undoubtedly the source of the quinoid compounds. A rapid test for alpha dicarboxyls in fats and oils is described. J.L.H.

76. The Antioxidative Behavior of Vegetable and Animal Fats. CALVIN GOLUMBIC, Bio-Chem. Lab., State Univ. of Iowa, Iowa City. *Oil and Soap*, 20, No. 6: 105-107. June, 1943.

Autoxidizing animal fats exhibit a well-defined induction period, the end of which coincides with the development of oxidative rancidity. Vegetable fats, however, do not have a sharply defined induction period and they show oxidative rancidity before the period of relatively rapid oxygen uptake and peroxide formation.

A kinetic study of the oxidation of tocopherol during the induction periods of animal and vegetable fats yielded information on possible causes of the differences in the induction periods. The oxidation of tocopherol in both fats is accompanied, in the early stages, by the formation of tocoquinones. In animal fats the complete disappearance of added tocopherol marks the end of the induction period and the beginning of organoleptic oxidative rancidity.

Chroman, -5, 6-quinones appear during the course of the induction period of autoxidizing vegetable, but not animal fats. These O-quinones, derived from unknown precursors, are antioxidants and their relatively slow oxidation rate as compared with tocopherol permit them to act after the

disappearance of tocopherol. This action offers an explanation for the absence of sharp induction periods of vegetable fats.

Tocopherols are decreasingly effective as antioxidants when employed at higher levels. This accounts for the previously recognized ineffectiveness of tocopherols and inhibitol concentrates when added to vegetable fats.

J.L.H.

77. **Estimation of Vitamin A in Food Products.** BERNARD L. OSER, DANIEL MELNICK, AND MORTON PADER, Food Res. Labs., Inc., Long Island City, N. Y. Jour. Indus. and Engin. Chem., Analyt. Ed., 15, No. 12: 724. Dec., 1943.

Modification of the antimony trichloride method for determination of vitamin A in food products, including dairy products, was made. Corrections are allowed for the presence of inhibitors of the color development, for temperature effects, for variations in the reagent, for turbidities produced in the course of the color development, and for extraneous color present in the final test solution. The unreliability of the direct spectrophotometric method for the assay of foods is demonstrated. The reaction of carotene with antimony trichloride was studied.

B.H.W.

78. **Factors Affecting the Stability of Cottonseed Oil. A Study of the Antioxygenic Activity of Alpha-Tocopherol.** C. E. SWIFT, W. G. ROSE, AND G. S. JAMIESON, Bur. of Agr. Chem. and Engin., U.S.D.A., Washington, D. C. Oil and Soap, 19, No. 10: 176. 1942.

The results secured suggest that the antioxygenic activity of the tocopherols is due to their reactivity towards the active peroxides. The rate and extent of peroxide accumulation during the induction period was found to be dependent on the tocopherol content of the oil. The tocopherols function most effectively at lower levels of concentration and with decreasing efficiency at higher levels. Small amounts of the cephalin fraction markedly retarded the rapid initial rate of oxidation of  $\alpha$ -tocopherols. This effect demonstrates the synergism of an "acid-type" substance acting with a "phenolic-type" antioxidant.

J.L.H.

79. **The Oven and Aeration Methods as Means of Accelerating Fat Oxidation.** F. C. EWBANK AND I. A. GOULD, Dept. of Dairying, Mich. State Col., East Lansing, Mich. Oil and Soap, 19, No. 12: 205. 1942.

A comparison was made of the aeration and hot oven methods of accelerating butter oil oxidation at 100° C. The rate of peroxide formation was used in determining the induction period; a peroxide value of 5 being arbitrarily chosen as the end of the induction period. The two methods were

found to be equally reliable when careful technique was employed and where the influence of certain variable factors were controlled. With the oven method especial attention must be given to temperature control, air agitation and arrangement of the samples in the oven if uniform results are to be obtained. The oven method gave a shorter induction period than the aeration method but the extent of oxidation over a relatively long period was less.  
J.L.H.

80. **The Antioxygenic Action of Phosphoric Acid in Association with Tocopherols and Hydroquinones.** CALVIN GOLUMBIC, Bio-Chem. Lab., State Univ. of Iowa, Iowa City, Ia. *Oil and Soap*, 19, No. 10: 181. 1942.

Phosphoric acid was found to retard the oxidation of tocopherol in autoxidizing fats and thus to increase its antioxygenic activity. The stabilizing capacity of hydroquinones was likewise found to be increased by small amounts of phosphoric acid. The data secured suggest that this synergistic action is due to the shifting to the left, of the hydroquinone  $\rightleftharpoons$  quinone equilibrium. The tocohydroquinone  $\rightleftharpoons$  tocoquinone equilibrium is a special case of this relationship in which phosphoric acid also catalyzes the cyclization of tocohydroquinone to tocopherol, thus regenerating the antioxidant.  
J.L.H.

81. **Vitamin A Added to Fats as Related to Stability During Baking.** E. E. RICE, H. C. BLACK, G. T. CARLIN, AND H. E. ROBINSON. Swift & Co., Chicago, Ill. *Oil and Soap*, 19, No. 9: 164. 1942.

The Carr-Price color reaction and U.S.P. bio-assays were used to determine the stability of vitamin A in various types of baked goods prepared with fats fortified with vitamin A. In bread, biscuits and cake which are relatively low in fat and baked under moderate conditions it appears that 80 to 100% of the vitamin survives the baking process. When the fat content is higher and the baking conditions more severe as in pie crust, considerable vitamin A destruction is likely to occur depending on the extent of the baking.  
J.L.H.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

82. **The Nitrogen Distribution in Dried Milk.** U. S. ASHWORTH AND HARRIS O. VANORDEN, Div. of Dairy Hus., Agr. Expt. Sta., Pullman, Wash. *Jour. Milk Technol.*, 6, No. 5: 272. Sept.-Oct., 1943.

Samples of spray-dried skimmilk were analyzed to determine whether the nitrogen distribution differed from that of normal skimmilk.

The results obtained when calculated to a moisture-free basis were found to agree very well with those reported in the literature for fresh skimmilk

with the exception of the albumin-plus-globulin fraction. Out of 32 samples tested only four contained measurable amounts of this fraction. The amount found, no doubt, was associated with the heat treatment that the samples received prior to drying. L.H.B.

83. **A Laboratory Spray Drier.** A. H. WOODCOCK AND H. TESSIER, Natl. Res. Labs., Ottawa. *Canad. Jour. Res.*, A, 21, No. 9: 75. 1943.

A laboratory model of a cyclone-type spray drier which in operation has produced dried milk and dried egg of good commercial quality is described. The main drying chamber is an inverted cone 2 feet in diameter and 5 ft. 2 in. in height made of galvanized metal and covered with insulating materials. Adjustments are provided for inlet air temperature, total air flow, angular velocity of air in the drying chamber, quantity of liquid introduced, and spraying technique. The air is supplied by a fan similar to the type used in a large household vacuum cleaner and is heated by a thermostatically controlled, 5000-watt electric element. The maximum capacity is about four liters of liquid per hour. O.R.I.

## DISEASE

84. **Newer Methods for the Control of Bovine Mastitis.** G. R. SPENCER. Dept. of Vet. Sci., Univ. Wis. *Milk Dealer*, 33, No. 1: 52. Oct., 1943.

To control the spread of mastitis by the dairyman, the infected animals must be "spotted" "by the strip cup and the Hotis test." Both tests are needed to determine both new or "carrier" cows. Segregation is recommended in lieu of slaughter at the present time as a means of preventing spread of infection. Segregated animals should be milked and handled by special personnel if possible, and if not, every sanitary precaution exercised to prevent spread of infection such as reduced feeding to facilitate treatment; thorough milking of all quarters; avoidance of teat injuries and specific medicinal treatment to cure and reduce extent of infection, are recommended as control measures. C.S.T.

85. **Production and Prevention of Bloat in Cattle on Alfalfa Pasture.** H. H. COLE, S. W. MEAD, AND W. M. REGAN, Univ. Calif., Davis, Calif. *Jour. Anim. Sci.*, 2, No. 4: 285-294. Nov., 1943.

Bloat was produced at will in dairy cows by depriving them of hay and bedding for at least 48 hours and pastured on thick stands of immature alfalfa that was 8 to 14 inches high. A thick stand makes possible rapid ingestion. It was necessary to use pastures free from weeds and contaminating grasses. Cows tend to select considerable amounts of coarse, seemingly unpalatable weeds where succulent alfalfa is abundant.

The availability of the water in the pasture did not affect the incidence of bloat. The feeding of alfalfa hay reduced the incidence and severity of bloat but was not completely effective. Bloat was effectively controlled on alfalfa pasture by feeding Sudan hay in the corral or in the pasture. Also, pasturing on Sudan at night before placing the cows on alfalfa pasture appeared to be an effective means of controlling bloat. Cows ruminated only one-half as much on alfalfa pasture as on Sudan pasture. Apparently both rumination and belching are readily induced by Sudan because of scabrous leaves. C.F.H.

**86. A Cheese-Borne Epidemic of Typhoid Fever.** JACQUES GAUTHIER AND A. R. FOLEY, Min. of Health and Soc. Welfare, Quebec. *Canad. Jour. Pub. Health*, 34, No. 12: 543. 1943.

An outbreak of typhoid fever which resulted in 40 cases and six deaths is described. Evidence indicated that milk supplied to the cheese factory became contaminated from a carrier working on a farm. The cheese was of the Cheddar type and was consumed when about ten days old. Butter made in the same factory from pasteurized cream was not incriminated. In addition to the control measures taken the following recommendations are made:

1. Early notification of each case of enteric fever.
2. General check-up of typhoid carriers.

3. Pasteurization of the cheese milk or at least the holding of the product for a three-month period before consumption. O.R.I.

**87. Effects of Disease on Nutrition. I. Absorption, Storage, and Utilization of Vitamin A in the Presence of Disease.** SAMUEL SPEC-  
TOR, CHARLES F. MCKHANN, AND EMILY R. MESERVE, Dept. of Ped.  
and Communicable Dis., Univ. of Mich. Med. School, Ann Arbor,  
Mich. *Amer. Jour. Dis. Children*, 66, No. 4: 376-395. Oct., 1943.

This paper is a review of the effects of disease on absorption and utilization of vitamin A. Most of the data cited were obtained with human subjects, and the review has been made from the viewpoint of human medicine. The effects of several pathological conditions are discussed, which includes jaundice, celiac syndrome, cystic fibrosis of the pancreas, infection, cirrhosis of the liver, valvular lesions of the heart, malignant neoplastic tissue, disorders of the thyroid, and allergy.

It is pointed out that malnutrition may be the result of organic disturbances within the patient. For example, during infections there develops rather regularly a decrease in the ability of the intestinal tract to absorb carotene and vitamin A. This decreased absorptive power occurs even with infections that do not primarily involve the gastrointestinal tract.

It is not possible to make a complete review of this article, as it in itself is a review. Sixty-two references are given. R.K.W.

## FEEDS AND FEEDING

88. Feeding Standard Equation for Cows and Goats in Milk. W. L. GAINS, Univ. Ill., Urbana, Ill. Jour. Anim. Sci., 2, No. 4: 304-313. Nov., 1943.

The precision data used in this investigation were reported in the literature from the Pennsylvania Agricultural Experiment Station. One hundred seven observations on 10 cows were used in the formulation of a standard equation for lactating cows. The feeding standard equation for the milking cow is as follows:

$DN = 0.008 W + 0.3 \text{ F.C.M.}$ , in which DN = daily digestible nutrients intake in pounds, W = live weight in pounds, F.C.M. = daily milk-energy yield in pounds 4% milk. C.F.H.

89. Studies on the Alimentary Tract of Merino Sheep in South Africa. VI. The Role of Infusoria in Ruminal Digestion with Some Remarks on Ruminal Bacteria. J. G. VAN DER WATH AND S. J. MYBURGH, Section of Biochem., Onderstepoort, Pretoria, South Africa. Onderstepoort Jour. Vet. Sci. and Anim. Indus., 17, Nos. 1 & 2: 61-85. Oct., 1941.

A technique is described for the preservation and counting of ruminal infusoria.

The changing of the ration of sheep from alfalfa and corn to corn alone resulted in extinction of the large type of infusoria and a reduction of all types following the cessation of rumination. Appetite of the sheep was adversely affected. A ration of wheat straw alone resulted in the starvation of ruminal infusoria. The addition of corn to the wheat straw resulted in a marked increase in the number of infusoria. The feeding of green alfalfa only brought about a reduction in the number of infusoria.

Season fluctuations of ruminal infusoria of grazing sheep are described. The amount of protein in pasture had a significant influence on the density of the infusoria population.

The rate of digestion of fine yellow corn meal by starved infusoria in the rumen was studied. Yellow corn particles were found in the infusoria one minute after being placed in the rumen. Disintegration of corn particles was observed within the infusoria. The so-called glycogen granules observed within the plasma of the infusoria were shown to be glycogen-synthesizing bacteria. The food and bacteria ingested by the infusoria were digested by enzymes secreted by the bacteria present. In an experiment where the infusoria and bacteria were destroyed by feeding copper sulphate and the rumen inoculated with starch-digesting bacteria, it was shown that infusoria are not necessary for starch digestion.

Infusoria engulfed particles of cellulose. The digestion of cellulose within the body of the infusorium is primarily due to cellulose-digesting bacteria ingested by the infusorium. C.F.H.

90. **Brown Silage from Atlas Sorgo—Chemical Composition and Apparent Digestibility as Determined by Feeding to Dairy Cows.** H. E. BECHTEL, F. W. ATKESON, AND J. S. HUGHES, Kans. Agr. Expt. Sta., Manhattan, Kans. Jour. Anim. Sci., 2, No. 4: 295-303. Nov., 1943.

Chemical analyses of five brown silage samples and one sample of high moisture chopped brown fodder were compared with those of normal green Atlas sorgo silage. Three silages were used in digestion trials. (1) Normal green silage from a wire and roofing paper silo in which maximum temperature during storage was 92° F. (2) Brown silage from sorgo bundle silage in which the maximum temperature during storage was 141° F. (3) Brown silage from a straw bale silo in which the maximum temperature during storage was 147.5° F.

The chemical analyses showed no appreciable difference between the brown and normal silages except for carotene and possible ash. The amount of carotene in the brown (high temperature) silages was very low.

There was a marked decrease in the coefficients of digestibility for all nutrients except ether extract in the brown silage. The degree of digestibility appeared to depend on the maximum temperature during storage. Protein was most affected, the apparent digestibility averaged 55% for normal silage, 23 and 4% for the two brown silages.

Brown silage was less palatable than normal silage. C.F.H.

91. **Biological Methods of Measuring the Protein Values of Feeds.** H. H. MITCHELL, Div. Anim. Nutr., Univ. Ill., Urbana, Ill. Jour. Anim. Sci., 2, No. 4: 263-277. Nov., 1943.

The evaluation of biological methods of measuring the protein values of feeds is discussed. Conditions considered essential in measuring protein values of feeds are as follows:

1. The measurement of protein utilization by studying the nitrogen economy of the animal.
  2. The measurement selected must involve the use of dietary protein in maintenance as well as in production.
  3. The imposition of comparable levels of protein feeding.
  4. The control of the food intake of comparable animals, which generally means the equalization of food consumption.
- C.F.H.

92. **Feeding Dairy Stock in Wartime.** H. S. WILLARD. Wyo. Agr. Expt. Sta. Bul. 263, 19 pages.

Although an experiment station publication this is primarily an exten-

sion bulletin. Information is furnished on the use and importance of hay and pasture in dairy production, correct apportionment of grain allowances under differing conditions, grain mixtures suitable for feeding with different kinds of roughage, and wartime production of dairy calves and heifers. Several tables are included for the guidance of the stockman. J.G.A.

93. **The Influence of Dietary Fat of Varying Unsaturation on the Component Acids of Cow Milk Fats.** T. P. HILDITCH, Dept. of Indus. Chem., Univ. of Liverpool, AND H. JASPERSON, Res. Dept., Messrs. J. Bibby and Sons, Ltd., Liverpool. *Biochem. Jour.*, 37, No. 2: 238-243. 1943.

In most feeding trials wherein different oils or fats have been fed to dairy cows the results have been interpreted from the over-all or gross change in the degree of saturation of the butter fat produced, but in this particular study an attempt was made to differentiate between the component fatty acids responsible for this gross change.

Fifteen cows were divided into five groups and fed (1) a basal diet; (2) basal diet plus peanut oil which is highly unsaturated, having an I.v. (iodine value or iodine number) of 88; (3) basal diet plus partially hydrogenated peanut oil having approximately the same I.v., 45, as soft butter fat; (4) basal diet plus hydrogenated peanut oil which was almost completely saturated—I.v. of 17; and (5) basal diet plus palm kernel oil which is naturally highly saturated, I.v. of 17.

Groups (2) and (3) produced butter fat with an increased amount of oleo-glycerides and a decrease in the butyric to caprylic glycerides. Group (5) produced butter fat with the lauro-glycerides three-fold greater and myristo-glycerides 20% greater than the control, but the oleo- and palmito-glycerides showed a slight decrease. Group (4) receiving the highly hydrogenated or saturated peanut oil gave butter fat more nearly like the control group in its composition. This is explained on the basis that about half of the fat fed was so completely saturated that its melting point was above the body temperature of the cow and so was not assimilated. It is interesting to note that although the fats fed to groups 4 and 5 had the same I.v. they produced butterfats of quite different detailed fatty acid compositions. Peanut oil normally contains about 20% of the unsaturated acids, linoleic, arachidic, and lignoceric acids, but these did not pass into the butter fat. It seems that the mammary gland has a selective action in the absorption of oleic (as distinct from linoleic) acids.

A good review of previous work is given.

A.O.C.

94. **The Utilization of Urea in the Bovine Rumen.** 1. **Methods of Analysis of the Rumen Ingesta and Preliminary Experiments in Vivo.**



R. M. PEARSON AND J. A. B. SMITH, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. Biochem. Jour., 37, No. 1: 142-148. 1943.

"The relative merits of trichloroacetic acid, sodium tungstate with sulfuric acid, and alcohol have been compared as precipitants in the estimation of non-protein-nitrogen in rumen ingesta. A description is given of the methods finally adopted for the estimation of non-protein-nitrogen, urea and ammonia."

Samples of the rumen ingesta were taken for analysis from the rumen fistula of a steer, but the authors concluded that there were so many difficulties involved in the *in vivo* experiments, especially the fact that it was practically impossible to secure a representative sample, that "the results of *in vivo* experiments of this type cannot be regarded as supplying evidence either for or against the theory that urea is converted to protein in the rumen," and because of this, they state that *in vitro* methods would be more reliable.

A.O.C.

**95. The Utilization of Urea in the Bovine Rumen. 2. The Conversion of Urea to Ammonia.** R. M. PEARSON AND J. A. B. SMITH, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. Biochem. Jour., 37, No. 1: 148-153. 1943.

Samples of rumen ingesta taken from the gastric fistula of a steer were used in making *in vitro* studies of its action on urea.

All of the urea which would ever be likely to be fed to a cow will be converted to ammonia within an hour.

The urease preparation derived from the rumen was found very similar to the ureases from soya or jack beans in its behavior to temperature, pH and inhibitors. Preliminary attempts to obtain enzyme preparations free from bacteria proved unsuccessful.

A.O.C.

**96. The Utilization of Urea in the Bovine Rumen. 3. The Synthesis and Breakdown of Protein in Rumen Ingesta.** R. M. PEARSON AND J. A. B. SMITH, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. Biochem. Jour., 37, No. 1: 153-164. 1943.

The liquid rumen contents taken from a gastric fistula of a steer were studied *in vitro*. In order to avoid any appreciable change in the "microbiological picture" incubation periods of only 2 to 4 hours were used. Urea was added to the liquid before incubation. Most all of the non-protein nitrogen was made up of urea and ammonia. Synthesis of protein appeared to occur to the extent of about 9 mg. N/100 gms. rumen liquid. While the total nitrogen remained constant, there was a decrease in the non-protein nitrogen accompanied by a parallel decrease in the ammonia, suggesting that the protein synthesis was from ammonia rather than from urea. This

conversion of ammonia to protein is thought to be microbiological in nature. Accompanying the protein synthesis is a protein breakdown—and either may predominate depending upon the general conditions or the substances present. A.O.C.

97. Urea as a Partial Substitute in the Feeding of Dairy Cattle. E. C. OWEN, J. A. B. SMITH, AND N. C. WRIGHT, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. *Biochem. Jour.*, 37, No. 1: 44-53. 1943.

Results are given for feeding trials with seven lactating Ayrshire cows over a period varying from 100 to 160 days wherein a third of the nitrogen of the feed was given in the form of urea and then substituted for an equal amount of nitrogen in the form of blood meal.

"The nitrogen balance and excretion data show that, although urea was partially retained by all the animals, its retention was not complete. Compared with blood meal, amounts varying from some 12 to 47% and averaging 25% of the ingested urea apparently passed through the animal without being utilized. This apparent wastage was much reduced when urea feeding was preceded by a period in which the diet had been deficient in total nitrogen.

"The milk yields of five of the seven animals were well maintained when blood meal was replaced by urea. With four of the five cows under test a rapid and significant decrease in milk yield took place when urea was removed from the food."

Data for milk yield, body weight and nitrogen balance are given for the periods. There were no significant differences in the composition of the milk. A.O.C.

## FOOD VALUE OF DAIRY PRODUCTS

98. Dairy Products in the Wartime Dietary. H. H. MITCHELL, Prof. of Anim. Nutr., Univ. of Ill., Urbana, Ill. *Milk Dealer*, 32, No. 10: 28-30, 78-80. July, 1943.

Despite the fact that milk supplies a generous amount of essential nutrients, its value is in the variety of ways it may be used to serve different purposes on man's menu. The processing of milk into the wide variety of products to which it lends itself show the changes even in nutritive value to which it is adapted. As an example, Limburger cheese may increase two to three times in pantothenic acid, niacin and biotin due to the curing process. Fortified oleomargarine and butter are compared nutritionally. The author deplors the advent of the role medical men are taking in formulating nutritional policies to the exclusion of scientific research, since to the medical profession nutrition "is only one of a number of fields of interest" and therefore lead to deductions not subscribed to by trained nutri-

tionists. The fortification of bread with vitamins and the possible exclusion of milk-solids-not-fat is cited as an undesirable procedure since 6% of skim milk powder added to bread "improves the growth-promoting and bone calcifying value of the bread much more than does its enrichment with the official proportions of thiamin, niacin, and iron." Skim milk powder would improve the nutritive value of all bread. The shift to greater sale and use of whole milk from the farm as a wartime need is discussed in its relationship to supplanting skim milk for animal feeding purposes which "would raise serious obstacles in the production of pork, poultry and eggs" were it carried to an extent recommended by some economists. The efficiency of the dairy cow in converting concentrate grain into milk of greater nutritive value than the grain consumed is given as 70% as compared with the 72 to 75% efficiency of the miller converting wheat into patent flour of lower nutrient value than the wheat processed. C.S.T.

**99. Dairy Research and Human Nutrition.** O. E. REED, Chief of Bur. of Dairy Indus., Agr. Res. Admin., U.S.D.A., Washington, D. C. Milk Dealer, 32, No. 12: 35, 78-88. Sept., 1943.

Research in human nutrition is responsible for increased emphasis on the nutritive quality of food supplied to both our armed forces and civilians in this war, whereas quantity of food only was stressed in the last war. Dairy and animal research since the last war has made available information on what is needed to supply human nutritional needs, as to the kind and amount of nutrients required to maintain health, and the effect of lack of certain essential nutrient substances upon physical development. Research and surveys conclusively reveal the high place of milk as prerequisite for nutrition and health in the diet. The United States produces enough milk to provide a quart of milk per day per individual, but unfortunately we do not consume that quart in its entirety but instead utilize only the cream, the butterfat or the curd to the detriment of our nutritional welfare. The author traces the historical growth of the butter and cheese industry and inventions which made possible the complete utilization of the milk as it comes from the cow. The era of vitamins starting in 1912 and 1913 as the result of research gave an impetus to use of all the milk as well as to the respective merits of different vitamins. Nutritional research on vitamin A content of butter was started in 1941 and 23 states are cooperating in this nutritional survey. The "wealth of essential nutrients below the cream line" is likewise being studied. The study of the proteins, milk sugar and inorganic salts of milk but substantiate the importance of the nutritional role such ingredients play in an adequate diet, over and above the role vitamins of milk play. The role of still other constituents of milk wherein a growth factor has been shown is being studied and "every nutritive essential that we have discovered to date has been found to exist in the milk of

the cow. . . ." "Dairy products and good nutrition are inseparable. Good nutrition is fundamental for the physical, economic and moral progress of our people." By marketing the entire output of the dairy cow the dairy industry can best contribute to that progress. C.S.T.

100. War Needs Teach Nutritional Value of Milk to British Consumers.

GEORGE WALWORTH. *Milk Dealer*, 33, No. 3: 32-33. Dec., 1943.

Previous to the war, British fluid milk consumption averaged only one-third pint per day per capita, and efforts to subsidize milk for school children and general use largely failed. With the advent of war, however, the need for adequate nutrition and the recognition of milk as a basic food to prevent malnutrition of mothers, children and low-income groups was recognized by the British Government, and its distribution to such groups was subsidized and rationed on a preferential basis. The postwar effect of the recognition of the valuable nutritional role of milk and dairy products to the British consuming public should result in increased sale of all such products and serve as "the finest investment a government can make for the health and welfare of its people." C.S.T.

101. The Ratio of Ascorbic Acid, Riboflavin and Thiamine in Raw and

Pasteurized Milk. A. D. HOLMES, C. P. JONES, A. W. WERTZ, AND J. W. KUZMESKI, Mass. Agr. Expt. Sta., Amherst, Mass. *Jour. Nutr.*, 26, No. 4: 337-345. Oct., 1943.

Composite milk from a herd consisting of 18 Ayrshires, 13 Guernseys, 18 Holsteins and 11 Jerseys, collected during January, February and March was used in this study. The milk was held at 40° F. for an average of 10 hours prior to pasteurization in a stainless steel vat, pasteurized by the holding process for 30 minutes at 143-145° F.

The ascorbic acid content of the raw milk ranged from 14.0 mg. to 22.5 mg. and averaged 19.7 mg. per liter; after pasteurization, the extreme values were 7.0 mg. and 19.1 mg., with an average value of 15.9 mg. per liter. The loss of ascorbic acid during pasteurization was 18.3%.

The riboflavin content of the raw milk varied from 1.35 mg. to 1.75 mg. and averaged 1.51 mg. per liter. The corresponding riboflavin values for the pasteurized milk were 1.19, 2.06, and 1.48 mg. per liter, respectively. The loss of riboflavin during pasteurization was only 2%.

The thiamine content of the raw milk varied from 0.29 to 0.35 mg. and averaged 0.33 mg. per liter; after pasteurization, the extreme values were 0.21 and 0.34, and averaged 0.30 mg. per liter. The loss of thiamine during pasteurization was 9.1%. C.F.H.

102. The Application of Chromatography to the Study of the Carotenoids of Human and Cow's Milk. S. Y. THOMPSON, S. K. KON, AND

E. H. MAWSON, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. Abstracts of papers read at the Proc. of the Biochem. Soc., Nov. 7, 1942, London. Biochem. Jour., 36, Nos. 10, 11 & 12 (combined): xvii. 1942.

The authors "have chromatographed numerous samples of human milk fat and have found that  $\beta$ -carotene contributes from 20 to 25% or even less of the total absorption at 451 m $\mu$ . In the majority of samples examined a pink band lies above that of  $\beta$ -carotene, chromatographically homogeneous with and showing the same absorption curve as crystalline lycopene, obtained from tomatoes.

"Shorthorn butter fat showed no lycopene zone. . . . In Guernsey fat there were three absorption zones between those of  $\beta$ -carotene and xanthophylls, one of which was pink and occupied the lycopene position. When 80 pounds of fresh tomatoes were given to a Guernsey cow during 6 days, the intensity of these zones rose from 4 to 16% of the total absorption. The pigment was identified as lycopene. In Guernsey milk  $\beta$ -carotene forms a smaller proportion of the total pigments than in Shorthorn milk."

A.O.C.

## HERD MANAGEMENT

103. Dairy Management Problems. EARLE L. MOFFITT, Penn. State College. Milk Dealer, 32, No. 11: 32, 66-67. Aug., 1943.

Stating "that 95% of the success in operating a farm comes from the thought or headwork put into the business," the author stresses the factors essential for financial success. The need of accurate farm records, the use of the data such records reveal, and the careful account of all details mark the difference between success and failure. Farm management studies made by the Pennsylvania State College are cited that show that unless yearly farm sales equal at least 25% of the total farm investment, there is practically no chance to show a yearly profit. Values of \$15 to \$18 per acre of crops on a general farm and \$25 to \$35 per acre of crops on special farms are given as goals to attain if a profit is to be assured. Details of dairy farm management are itemized and the proper balance to maintain in accord with proven practices are cited. The need of proper allocation of time and effort to each phase of farm management is pointed out.

C.S.T.

## ICE CREAM

104. Dried Whole Egg Powder. VI. Effect of Storage Temperature and Gas Packing on Keeping Quality. W. HAROLD WHITE, M. W. THISTLE, AND MARGARET REID, Natl. Res. Labs., Ottawa. Canad. Jour. Res., D, 21, No. 9: 271. 1943.

Dried whole egg powders were obtained from three different manufacturers and stored at temperatures of 45°, 60°, 75° and 90° F. for periods up

to six months. Quality was assessed by determination of fluorescence and potassium chloride values. At 75° F. the rate of deterioration was comparatively rapid and at 90° F. it was markedly so. To maintain quality during storage dried egg should be stored at a temperature of 60° F. or lower.

The effect on keeping quality of packing in nitrogen, carbon dioxide, under vacuum, or in the form of compressed tablets was studied. Carbon dioxide alone had beneficial effect. O.R.I.

105. **Dried Whole Egg Powder. VII. Effect of Temperature and Moisture on the Bacterial Content of Liquid and Dried Egg.** N. E. GIBBONS AND C. O. FULTON, Natl. Res. Labs., Ottawa. *Canad. Jour. Res., D*, 21, No. 10: 332. 1943.

The bacterial content of liquid egg increased rapidly after about 6 hours at 68° F., 12 hours at 60° F., 25 hours at 52° F. and two or three days at 45° F. At 38° F. there was little change for five or six days, followed by a very gradual increase.

The bacterial content of the dried egg powder was influenced by the number of bacteria in the mélange, the drying temperature, the rate of cooling, the storage temperature and the moisture content. Low drying temperature and rapid cooling of the powder favored survival. On storage the bacterial mortality increased with increasing time and temperature. Up to 8.6%, moisture content had little effect on bacterial survival. At moisture levels above 5% there was an increase in the number of molds, particularly at 75° and 90° F. O.R.I.

106. **Acid Standardization.** A. D. BURKE, Ala. Polytechnic Inst., Auburn, Ala. *Ice Cream Field*, 43, No. 3: 64. 1943.

Early in the history of the ice cream industry it was considered good practice to "ripen" ice cream mixes and that a "livery" mix was believed to be the secret of good whipping, high yield and smooth-bodied ice cream. Experimenters early proved the fallacy of this concept and further showed that aging had little advantage beyond 24 hours.

The practice of adding certain salts to ice cream mixes as a basis of controlling mix viscosity, the author considers was the start of acid standardization. Five objections to "neutralization" are given but the author concludes that there are conditions which justify acid standardization or "neutralization."

Advantages listed for acid standardization are as follows: (1) provides uniform control of viscosity, (2) aids in whipping properties of high acid mixes, (3) improves melt down qualities of high acid mixes, (4) it helps intensify the apparent sweetness and (5) it may be of some benefit in retarding sandiness.

The author warns against over neutralization but considers it safe to reduce the acidity of ice cream mixes to about 0.18%. He suggests the following basis of calculating the correct value for acid standardization:

$$\frac{\text{Serum solids of mix}}{\text{Serum solids of milk}} \times \text{acidity of milk} = \begin{cases} \text{desired} \\ \text{standardized} \\ \text{acidity} \end{cases}$$

Directions are given for "neutralization" dependent upon weight of mix, its original acidity and the neutralizer used. W.C.C.

**107. Bugaboo of Barriers.** HAROLD F. PIERCE, London, Ontario. *Ice Cream Field*, 43, No. 3: 42. 1943.

Many of the practices necessitated by the war have been beneficial to the ice cream industry of Canada. Costs have been reduced because of territory exchanges, reduced dealer service and cabinet repairs as well as marked reduction in advertising it is claimed. Hope is expressed that certain of the advantageous practices will continue after the war.

"Bootlegging" has been a minor problem. Strict adherence to quotas has been practiced. Cereal substitutes for dried milk have been used and sugar substitutes such as honey and corn syrup are officially tabooed. Glucose, although still allowed, is practically unavailable.

Regret is expressed that advertising has been so drastically reduced. The author concludes that except for the question of good-will building, dealer supervision, advertising and dealer help the Canadian picture is satisfactory. W.C.C.

**108. What Other Solids to Use? The Serum Solids Situation.** C. C. FLORA, Va. Polytechnic Inst., Blacksburg, Va. *Ice Cream Field*, 43, No. 3: 19. 1943.

The author briefly discusses some of the problems resulting from wartime restrictions in the ice cream industry. He reviews the findings of various investigators and commercial operators as to what products can be used to replace serum solids in ice cream. He mentions a few of his findings which in general confirm those previously reported.

It is concluded that corn sugars, apple juices and syrups "offer possibilities for replacing part of the serum solids in ice cream." Oat flour (Avenex), Cincrose, soybean flour and wheat flour are also placed in this same category. W.C.C.

**109. Serum Solids Substitutes.** C. D. DAHLE AND D. V. JOSEPHSON, Pennsylvania State Col., State College, Pa. *Ice Cream Field*, 43, No. 3: 14. 1943.

Experimental mixes which contained 10.5% fat, 8.4% serum solids, 15% sugar 60% of which was Frodex and 0.4% gelatin were prepared with vari-

ous flours by pasteurizing them at 160° F. for 20 minutes, since this was found to give better results than heating at 150° F. for 30 minutes. The results obtained confirmed the findings of others and further showed that Avenex or oat flour when used to the extent of 1% "gave as good or better results than 2% of the others."

They conclude that when over 1% oat flour is used a cereal flavor results but 2% soya flour also imparts a cereal flavor, whereas 2% of wheat, cake, or corn flour "caused no particular flavor defect in vanilla ice cream." Even "3 or 4% of ordinary wheat flour gave little indication of cereal taste."

The authors report that nothing except egg yolk and monoglyceride was found to improve overrun.

It is recommended that mixes containing cereal products be well agitated when used to insure the proper dispersion of part of the cereal which settles during standing.

W.C.C.

## MILK

110. **Calculating Total Solids in Milk.** CHARLES W. LIVAK. *Natl. Butter and Cheese Jour.*, 35, No. 1: 13. Jan., 1944.

Samples of milk were taken in November, December and January and analyzed for fat and solids by the Mojonnier method. Specific gravity measurements were made with lactometers and a Westphal balance. Percentages of total solids were calculated by Sharp and Hart's equation. The total solids so calculated were 0.228 and 0.307% higher than the Mojonnier method indicated, depending upon whether Rueda's or a "theoretical" correction factor was used to convert specific gravity values to 86° F. with reference to water at 86° F.

W.V.P.

111. **Why Certified Milk Should be Retained.** J. HOWARD BROWN, Ph.D., Sc.D., Chairman, Council of the Amer. Assoc. of Med. Milk Commrs. *Milk Dealer*, 32, No. 10: 48-49. July, 1943.

The possibility of the elimination of certified milk by allowing only two grades of milk as a war measure is discussed and a plea is made for retention of "certified milk" as a medicinal food rather than a premium milk. Certified milk is controlled by the American Association of Medical Milk Commissions, Inc., a non-profit organization. This milk is not produced and marketed for financial profit and its methods and standards of production are rigorously controlled. Nutritional, chemical and sanitary differences are maintained as are special qualifications of herds licensed to produce such milk. Ninety per cent of the sales of certified milk are made upon the prescription or recommendations of qualified physicians who consider it as a product "which fills a need and exerts an influence far beyond what its



volume of production and consumption might seem to justify." Its continuation as such a distinct product is urged. C.S.T.

**112. Plant Cooling System for Milk Dealers.** ANONYMOUS. Milk Dealer, 32, No. 10: 32-33. July, 1943.

The adaptation of the cooling of water for refrigeration purposes to apply to flat-top dairy plants by use of a spray canopy system is recommended as a means of controlling and reducing plant building temperatures and as an auxiliary air-conditioning aid. The system advocated is by means of feed water pipes installed above the roof rather than under it, so that pipes require no insulation and can be operated with waste water from condenser coils at a nozzle pressure of 7 pounds. A spray pond covering the whole roof to protect it from solar heat, with a circulating system providing for recirculation, properly installed will reduce afternoon temperatures within the plant of a one-story flat-roofed building from 10 to 15 degrees. Such a system will also increase the life of the roof by stopping excessive expansion and contraction of roof material and loss of volatile oils from asphalt by hot sun. C.S.T.

**113. Making Quality Easy for Milk Producers.** PAUL H. MONDT. Milk Dealer, 32, No. 11: 30-31. Aug., 1943.

Farm labor as well as plant labor is critical. Therefore the producer needs all the help the plant operator can extend to promote continued production of quality milk. The producer should be educated as to the need of quality to prevent waste, promote edibility and use of dairy products, and to provide extra food. This can be done by means of posters, loaning such items as hair clippers, and by distributing whitewashing formulas and directions to farmers. Directions for the proper care of milking machines; blueprints on correct milk house construction, together with cement or building instructions; providing building forms for cooling tanks; aid in the purchase of supplies, cans, brushes, powders, etc.; and suggestions for the correct care of the cows, equipment and milk are discussed. C.S.T.

**114. Consumer's Problems of Every-Other-Day Milk Delivery.** GEORGE F. DOW. Milk Dealer, 32, No. 12: 33-34, 66-68. Sept., 1943.

The "other side," namely, the consumer's rather than the distributor's problems of every-other-day milk delivery is presented. Such factors as the keeping quality of milk, home refrigeration facilities and changes in source of supply are analyzed from a survey of 966 families in Portland and Westbrook, Maine. Consumer problems were souring of milk in hot weather, off-flavor development from standing outside or uncapped in home refrigerators, thickened cream layer, poorer keeping quality of raw milk as com-

pared with pasteurized, and the relation of time of delivery to the keeping quality of the milk delivered. The type of home storage of the milk was a factor in that ice refrigeration as compared with mechanical presented more of a quality and keeping problem. Detailed percentages of various complaints are given. On the whole the net change in milk purchased and consumed was negligible; quality was unaffected if adequate refrigeration was available in homes and if the time of delivery was adjusted. Seventy-six per cent received their milk from distributors with established home deliveries and but few changed dealers, thus demonstrating that consumers were willing to cooperate with the plan to conserve trucks and labor as a war measure. C.S.T.

115. **Pioneer Milk Pasteurizing Plant.** FRED W. MEEN. Milk Dealer, 33, No. 2: 39-40. Nov., 1943.

The description of equipment, methods used and of delivery and sales methods followed in the organization and starting of what is said to be the first commercial bottled fluid milk pasteurization plant established in the United States at Rush City, Minnesota, in 1897, is of historical interest to the milk industry. C.S.T.

116. **Securing Customer Cooperation to Ease Milk Delivery Problem.** C. W. ESMOND, G. P. Gundlach & Co., Cincinnati, Ohio. Milk Dealer, 33, No. 2: 48-50. Nov., 1943.

With the necessarily curtailed service to all milk customers in effect as a wartime emergency measure, the need for customer cooperation—always essential for best dealer-customer relationship—is now more than ever a “must” if confusion, complaints and loss of customers are to be avoided. An intelligent, systematic and continuous educational program to acquaint the customer with the problems confronting the dealer together with a recognition of the consumer’s unaccustomed inconveniences and problems will go far towards solving present-day wartime milk delivery problems, as well as to meet post-war competition and “to retain and to improve upon all the economical distribution methods of war time, such as the elimination of special delivery, standing orders, conservation of bottles, reduced number of deliveries per week, minimizing the overlapping of routes, collecting at lowest possible cost, saving unnecessary steps for milkmen, etc.,” and thus retain and maintain the closer and more friendly dealer-customer cooperation developed as a wartime necessity. C.S.T.

117. **Improving Chocolate Drinks.** B. E. HORRALL, Purdue Agr. Expt. Sta., Lafayette, Ind., AND M. O. MAUGHAN, Amer. Dry Milk Inst., Chicago, Ill. Milk Dealer, 33, No. 3: 24-25, 48. Dec., 1943.

A survey cited by the authors revealed that 80% of all chocolate milk sales was a “plus” not a “replacement” volume, indicating that chocolate

increases the palatability of milk to many consumers. Studies therefore were undertaken "to (a) improve the flavor and (b) increase the nutritive value of chocolate drinks." Experimental technique was to use various percentages of milk fat where milk solids-not-fat were added to different lots of whole and separated milks plus four different chocolate products and sugar. Cream, butter and butteroil were used for desired fat combinations and spray-dried skim milk powder for solids-not-fat additions. Six experiments were conducted with varying percentages of milk fat to which added milk solids-not-fat were combined. All samples were tested by seven or more judges. Added milk solids-not-fat to the extent of 3% improved all commercial chocolate milk drinks. A 2% butterfat product enriched with 3% milk solids-not-fat was adjudged the best-flavored product, followed closely by the 3% and 1% butterfat products to which 3% milk solids-not-fat were added. A commercial formula for addition of spray-dried skim milk powder to either a skim milk or reconstituted milk mixture is given.

C.S.T.

**118. The Function of the Laboratory in the Control of Milk Supplies.**

F. W. FABIAN, Res. Prof. Bact., Mich. State Col., East Lansing,  
Mich. Jour. Milk Technol., 6, No. 5: 278. Sept.-Oct., 1943.

A good discussion is given of the various laboratory tests used in the dairy industry and their relative values in determining the sanitary quality of milk.

The author concludes that "it looks as though we are going to use the plate method less since it is expensive, time-consuming and tells only a part of the story. In its stead we shall substitute the direct microscopic test supported by other tests. For raw milk, we shall use the odor test, the sediment test, and either the direct microscopic or the resazurin test or both. For pasteurized milk, we shall use the direct microscopic method supplemented by the coliform and phosphatase tests."

The author believes it will be some time yet before laboratory tests will be developed to the stage where they will supplant the milk inspector. He does think, however, that the future inspector will be better trained and will be a combination inspector and laboratory man, doing most of his own laboratory work.

The ideal situation is to use the milk inspector to locate the visible dirt and the laboratory to find the invisible dirt. "They should supplement rather than supplant each other."

L.H.B.

**119. Care of Milking Machines.** C. K. JOHNS, Central Expt. Farm,  
Ottawa, Canada. Jour. Milk Technol., 6, No. 5: 274. Sept.-Oct.,  
1943.

In studies made at the Central Experiment Farm, it was found possible to keep milking machine rubber parts in good sanitary condition without

the use of hot water after each milking. This method was developed in 1930 and has been in use ever since.

During a period of nearly twelve years, more than 1200 samples of the mixed raw milk (nights and mornings) were taken from the pasteurizing vat prior to pasteurization. More than 70% of the agar plate counts were below 10,000 per ml. and more than 95% were below 50,000 per ml.

The method used is very simple and is as follows:

Immediately after milking at least two gallons of clean, cold or lukewarm water are drawn through each unit, raising and lowering the teat cups several times to obtain an air-brush effect. The outer surfaces of the teat cup assembly are then cleaned off with a brush and hot detergent, hung on a solution rack and filled with a weak (0.5%) lye solution until the next milking. At that time the solution is drained out and the units re-assembled and ready for use. It has not been found necessary to rinse the units to remove traces of lye solution, but a chlorine rinse at this time could be drawn through the unit to advantage. At intervals of one or two weeks, the rubber parts are dismantled, primarily to prevent adherence to the metal, and for thorough inspection of the tubing and liners to note their condition. Worn out tubing and liners are replaced at this time, and if necessary, liners are trimmed to proper length. The tubes and liners are brushed in hot detergent solution and rinsed before re-assembling.

The lye solution has an advantage over the chlorine soak solution in that the lye saponifies any butterfat and dissolves any casein which might be present, thus leaving the rubber parts physically clean. The lye solution is also a good germicide and prevents the growth of bacteria in the tubes.

L.H.B.

## MISCELLANEOUS

120. Proper Paint Helps Solve Surface Maintenance Problems. ANONYMOUS. Milk Dealer, 32, No. 11: 29-58. Aug., 1943.

Stressing the need of proper maintenance of all milk plant equipment, the paint-up program, with paint still available, can and should be regularly maintained, particularly since the milk plant must of necessity operate under conditions of moisture and steam condensation especially destructive to ordinary paint.

The need of special moisture and acid-resistant protection from corrosion and fungus and mold growths by use of paints possessing such properties is stressed. The use of proper white and light-tinted paints for increased illumination which reduces eye strain and fatigue, aids employee morale and decreases accidents. In some plants accidents have been eliminated 100% by selection of a high light-reflecting paint. A table of reflection values for different colors ranging from 11 to 25% for dark green to 80-89%

for white is given. The suggestion is made that wherever practical, increased light reflection is obtained by painting floors white or light colored.  
C.S.T.

121. **First Aid for Ailing Cold Storage Rooms.** ANONYMOUS. Milk Dealer, 32, No. 11: 26. Aug., 1943.

Citing a recent survey made by the Refrigeration Service Engineers Society that approximately half of the refrigeration service men have left their former jobs, the author stresses the need for all fluid milk plants to keep their refrigerating machinery and cold storage rooms at top efficiency lest spoiled products through breakdowns with resulting higher operating costs result. A new type of circulating fan which requires no skilled mechanic to install provides for better air circulation on a new principle of blowing the cold air from the refrigerated coils up to the ceiling, down the walls and up the center, moving all the air in the room to maintain an even and equalized temperature. Such a movement of air promotes dryness, dispels odors quickly, prevents frosting of coils, retards mold growth and reduces cost. A diagram of air currents after installation of fan graphically illustrates diffusion of air.  
C.S.T.

122. **National Control or Free Competition for Britain's Milk Industry.** GEORGE WALWORTH. Milk Dealer, 33, No. 2: 64-66. Nov., 1943.

Whether government control of Britain's milk industry after the war is made permanent or whether the industry is permitted to revert to pre-war free competition is contingent upon the extent to which the present essential processing and price-control measures are voluntarily retained by private enterprise. To insure national nutritional requirements, proper price relationship as to milk quality and its utilization, the maintenance of proper balance between liquid milk and processed products consumption, economical maintenance of sales and distribution and milk quality control are factors the British milk industry must face and solve satisfactorily to avoid national post-war control of milk. The establishment of a milk control council by the industry with power to regulate such factors in the interest of consumers, as well as to insure adequate returns to the industry, is advocated. This British question is of importance to the United States in that post-war international relationships are involved and concern all milk-producing nations. "Liquid milk occupies the key position in Britain's agriculture but dairy produce will be of considerable significance in overseas trading with Britain."  
C.S.T.

123. **Use of Chlorine on the Farm.** JACK KEENAN, Gen. Lab. Div., Penna. Salt Mfg. Co., Philadelphia, Pa. Milk Dealer, 33, No. 2: 52-57. Nov., 1943.

Since milk quality control starts at the farm, the utilization of chlorine

for cleaning all utensils, for washing cows and milkers' hands, rinsing cans, etc., is of vital importance. Methods of preparing standard chlorine solutions and rinses are given for all such uses. Stress is laid upon need of using chlorine in washing cows' udders and teats to prevent infection, and in cleaning milking machines to prevent spread of disease. The author concludes that "next to sunshine, chlorine may be said to be our best bactericide" and that its use is safe, inexpensive and particularly adapted to use on the farm. C.S.T.

124. **Plastics.** ANONYMOUS. *Milk Dealer*, 33, No. 3: 26-27. Dec., 1943.

With the advent of war, substitute materials for use in dairy plant equipment became necessary. Since then substitutes for substitutes have appeared. The use of plastics, since they are light, unbreakable, clear and impart no flavor or odor, in making both old and new milk plant equipment, would seem to afford highly interesting possibilities. Particularly interesting is the possibility of supplanting standard tubing and piping with plastic material. C.S.T.

125. **Tin in the Dairy Industry.** JULIA DEXTER, Battellé Memorial Inst., Columbus, Ohio. *Milk Dealer*, 33, No. 3: 34, 46-48. Dec., 1943.

The use of tin in the dairy industry from the tin milk pail of the farmer to the tin can on the grocery store shelf through all the intermediate steps of collection, transportation, cooling, storing and packaging in tin-coated equipment is described and its present critical status discussed. New methods of utilizing tin as a coating which will favor retention of tin in postwar use in the dairy industry are described. Electrolytic plating of tin by different new methods; the use of white bronze plating to tin copper or in combination with nickel, tin spraying and new and better tinfoil for wrapping dairy products are postwar possibilities which "point the way to better and far heavier tin coatings than have been possible in the past. Repairs and relining are facilitated in many cases by electrolytic tinning means. Tin-coated steel is the most inexpensive material of construction for dairy equipment." C.S.T.

126. **Steel for Insulation.** JAMES G. MACORMACK, Amer. Flange & Mfg. Co., Inc., and C. T. Hogan & Co., Inc., New York. *Ice Cream Field*, 43, No. 3: 34. 1943.

The author claims that Ferro-Therm steel insulation offers increased efficiency and economy to the ice cream industry. Ferro-Therm steel insulation sheets are made of thin gauge commercial steel with a hot dipped lead and tin alloy coating "which reflects 90 to 95% of radiant heat. These sheets are installed with approximately one-half inch air space on either

side, broken up by separators every 24 to 32 inches. Ferro-Therm steel insulation was first introduced in 1933-34 but it has been extensively used since 1937 in many industries requiring refrigeration.

Eight sheets of Ferro-Therm insulation in an overall thickness of 4 inches is equivalent to 8 inches of corkboard. Ferro-Therm sheets from 26 U.S.S. gauge to 18 U.S.S. gauge are used for the outside linings for rigidity, while all intermediate steel sheets are No. 38 U.S.S. gauge and weigh 0.25 pound per square foot.

Fifteen sheets within a thickness of 6 inches used on a "dry ice" storage cabinet resulted in a loss of 1.8% for 24 hours whereas 8 inches of corkboard insulation on the same type of cabinet resulted in 3% loss per 24 hours. The Ferro-Therm container weighed 1100 pounds and held 2938 pounds of dry ice, whereas the corkboard container weighed 1500 pounds and held 2250 pounds of dry ice.

The degree of luster or optical brightness has little to do with the ability of the surface to reflect heat or infra red rays. Tests by the U. S. Bureau of Standards show that reflective insulating sheets exposed to industrial atmosphere for two years until they became a dull gray-black had 97% of the reflectivity of radiant heat they had when new and bright. Moisture condensation is very slight in a Ferro-Therm construction. The heat storage capacity of the steel insulation is relatively low, which permits more rapid temperature pull down than with the usual type of mass insulation.

W.C.C.





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## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

127. Further Studies on Coliform Bacteria Serologically Related to the Genus *Salmonella*. P. R. EDWARDS, W. B. CHERRY, AND D. W. BRUNER, Ky. Agr. Expt. Sta., Lexington. Jour. Infect. Dis., 73, No. 3: 229-238. Nov.-Dec., 1943.

In a continuation of work reported earlier (Jour. Dairy Sci., 25, No. 6: A144. June, 1942) the serological properties of 44 coliform cultures were studied. All of the cultures studied were considered pathogenic. An antigenic analysis revealed that the 44 cultures were divisible into 14 serological types and these types were divisible into 11 somatic groups. With the exception of 3 cultures, the H antigens of all of the types resembled those of *Salmonella düsseldorf* and *S. cerro*. The H antigens of the other 3 cultures resembled those of *S. enteritidis*. Antigenic formulas, based in part on the Kaufmann-White schema for the salmonellas, are given for the 14 types.

J.F.C.

### CHEESE

128. Consistency of Cheese Curd at Pitching and Grinding. F. M. V. COPPEN. Dairy Indus., 8, No. 9: 488. Sept., 1943.

The author discusses a number of control tests which are employed at different stages in Cheddar cheese manufacture. Data are given and the relative merits of these tests presented.

D.V.J.

129. Oil Separation in Processed Cheese. J. L. PALMER and W. H. SLY. Dairy Indus., 8, No. 8: 427. Aug., 1943.

In the manufacture of processed cheese much can be done to avoid oil separation during storage and distribution. It is pointed out that the ripeness of the cheese used for processing is a very important factor. Very ripe cheese frequently results in loose, grainy and oiled-off processed cheese while very fresh cheese produces a stable emulsion.

The stabilizing salt (called melting salt in England) is also a very important factor and according to these authors sodium citrate alone or a suitable blend of sodium citrate and phosphates produce the best results. Sodium metaphosphate is of questionable value. When the pH of a processed cheese is over 5.8 to 6.0 or below 5.4, there is more danger of oil separation. It is also pointed out that prolonged or violent agitation resulting in aeration will have a tendency to break down the emulsion. A continuous process is more desirable than a batch system.

D.V.J.

## DISEASE

130. Studies on Epidemic Diarrhea of the New-Born: Isolation of a Filtrable Agent Causing Diarrhea in Calves. JACOB S. LIGHT, M.D., AND HORACE L. HODES, Lt. MC, USNR, Johns Hopkins Univ. and Sydenham Hosp., Baltimore City Health Dept., Baltimore, Md. Amer. Jour. Pub. Health, 33, No. 12: 1451-1454. 1943.

In connection with four separate epidemics of diarrhea of the new-born a filtrable agent has been isolated which regularly produces diarrhea in calves. In the attempts so far made, this agent has not been isolated from the stools of normal infants or normal calves. The evidence suggests, though it is not conclusive, that the agent may be a cause of epidemic diarrhea of the new-born. M.W.Y.

131. Use of Modern Laboratory Aids in the Investigation of a Typhoid Fever Outbreak. EDWARD R. SCHLESINGER, Asst. Dist. Health Officer, N. Y. State Dept. Health, Albany, N. Y. Amer. Jour. Pub. Health, 33, No. 10: 1257-1262. 1943.

An outbreak of 27 cases of typhoid fever is described which occurred in Oswego County, New York, in 1941 and 1942. Twenty-three of the cases were probably due to the ingestion of curd or fresh cheese at a local factory or were secondary to such cases. The cases occurred over a period of 17 months. Bacteriophage typing separated the type C cases, due to the contaminated curd and cheese from type A cases traced to food prepared by two type A typhoid carriers. Two typhoid carriers were found among the dairy farmers supplying the cheese factory. Typing showed one to be type C and eliminated the other as the possible source of the outbreak, despite the fact that epidemiological evidence alone pointed more forcefully to the latter as the carrier responsible for the outbreak. M.W.Y.

132. What Is Known on Undulant Fever. K. G. WECKEL, Assoc. Prof. of Dairy Indus., Univ. Wis. Milk Plant Monthly, 32, No. 7: 41-42. 1943.

The disease "undulant" fever, deriving its name from its characteristic of recurring at intervals or in cycles, is known also as Brucellosis, intermittent typhoid, Malta or goat fever or subcontinuous fever depending in part on locality and observations. The disease has its origin in animals. The bacteria responsible for it among cattle is known as *Brucella abortus*; among sheep and goats as *Brucella melitensis*; and among swine as *Brucella suis*. Any type may cause undulant fever in man but that from *Brucella melitensis*, which has seldom been diagnosed in man in the United States, is the most fatal. *Br. abortus* are discharged through uterine secretions or, when the udder tissue is infected, through the milk. Humans consuming

raw milk may contract the disease but relatively this seldom occurs. The eradication program has reduced the incidence of the disease to a low of probably 2% or less. Three general methods are used in eradication: (1) Test and slaughter, (2) Test and segregation, and (3) Calfhood vaccination.

The incidence of undulant fever in humans is greatest among handlers of livestock and slaughterhouse or meat packing plant employees. The disease among humans is not contagious. There have been but very few recorded outbreaks of Brucellosis traced to milk and those to raw milk. No outbreaks have been reported from consuming pasteurized milk. Hence, the program of eradication and pasteurization minimizes the economic loss caused by the disease on farms and assures the consumer of a safe milk supply.  
G.M.T.

## FEEDS AND FEEDING

### 133. Physiological Requirements and Utilization of Protein and Energy by Growing Dairy Cattle. E. G. RITZMAN AND N. F. COLOVOS. N. H. Agr. Expt. Sta. Tech. Bul. 80. 59 pages. Aug., 1943.

An intensive study on the energy and protein requirements for growth of dairy cattle, from birth to approximate maturity. Eleven pure bred Holstein heifers were used in the following age groupings: Birth to 4 months, 3 heifers; 4-36 months, 4 heifers; 8-27 months, 4 heifers.

This bulletin lacks an adequate summary and it is difficult at times for the reader to distinguish between conclusions drawn from original data, and statements based on other work having a bearing on the results. The following statements, for the most part direct quotations, have been selected as being of particular significance.

A very close agreement was found between individuals in their basal metabolism at approximately the same age. A high pitch of tissue activity prevailed for about a week after birth; this was followed by a sudden drop, after which further decline was quite steady and gradual. This declining rate in tissue activity was reflected by a periodical decline in growth rate as indicated by daily gains in live weight in relation to total weight of the individual.

Certain of the results demonstrated the critical importance of the time factor in growth. Aging is almost purely a question of time, and maturity is reached in the span allotted for the species whether food is scarce or abundant.

An offshoot from the main investigation was a study of the effect of accessory illumination on metabolism. The conclusion that seemed most probable was that any effect of visual light on metabolism is immediate and prevails only as long as the (nerve) exciting effect of light is present.

The biological value of protein depends not only on the character of its nitrogenous constituents, or on the level of the protein intake, but also on

the physiological adaptability of the individual to utilize it. . . . The true measure of value of a feed for a given purpose must be determined by the individual which uses it most efficiently. The results do not suggest any beneficial results from the so-called associative effects of protein from different sources, but rather a low order of biological value of cereal proteins for growth.

Protein utilization values secured indicate that the protein of hay had a surprisingly high value for growth (practically the same as grain); that corn had a low value, that linseed meal was somewhat better than cottonseed meal, and that barley was about equal to oats.

Efficiency of protein utilization was determined as much by the inherent growth rate potential of the individual as by the available supply of food protein.

Relatively great differences may exist in protein utilization between individuals of similar age and type or breed, and maintained under like conditions.

The comparative uniformity of the decline in protein conversion indicates that it is impossible to force growth beyond these declining inherited limits.

The influence of hereditary limitations to storage of protein for growth appears to be the deciding factor as to the form in which available food energy in excess above maintenance is stored by the body.

Quantitative statements of requirements of protein and energy appear in detail in a series of tables which do not lend themselves to a simple summary statement. The reader is therefore referred to the original article for further specific information.

17 tables, 43 literature references.

J.G.A.

134. **Meeting the Protein Requirements of Dairy Cows.** H. B. ELLENBERGER. Vt. Agr. Expt. Sta. Pamphlet No. 1. 12 pages. March, 1943.

A non-technical discussion of the subject in the light of the present protein shortage. The need for good pasture supplemented by a simple grain mixture such as corn and oats, is stressed. The writer's opinion is that during the present emergency a concentrate mixture containing not more than 16% protein, if liberally fed, should serve most dairy needs, including winter feeding. The importance of storing ample supplies of roughage of the highest possible quality is strongly emphasized.

J.G.A.

## FOOD VALUE OF DAIRY PRODUCTS

135. **Milk in the National Food Program.** W. E. KRAUSS, Chief, Dairy Dept., Ohio Agr. Expt. Sta., Wooster, O. Milk Plant Monthly, 32, No. 9: 34. 1943.

Emergency food problems involve an adequate supply of calories, pro-

tein, minerals and vitamins from sources people have been accustomed to using and in keeping with the agricultural system which makes efficient use of land labor and feed. In 1942, about 40% of the food energy, 57% of the protein and 73% of the fat were from livestock products. Three-fourths of the calcium and about one-half of the riboflavin in the civilian food supply were from milk products. Considering the output of calories and protein from concentrates fed, the utilization of labor for the production of protein, the protein obtained per acre, the minerals and vitamins, milk ranks at the top followed closely by eggs. Milk and egg production are, therefore, emphasized. Indications are that the demand for milk may be maintained or even increased. G.M.T.

136. **Chemical and Biological Stability of Crystalline Vitamins D<sub>2</sub> and D<sub>3</sub> and Their Derivatives.** W. HUBER AND O. W. BARLOW, Res. Labs., Winthrop Chem. Co., Inc., Rensselaer, New York. *Jour. Biol. Chem.*, 149, No. 1: 125. July, 1943.

All forms of Vitamin D, including Vitamin D<sub>2</sub> (irradiated ergosterol) in a crystalline form, or when absorbed on casein (ertron), Vitamin D<sub>3</sub> (activated 7-dehydrocholesterol), as well as Vitamin D from natural sources, are susceptible to destruction through oxidation as shown by changes in their melting points and specific rotations. This decomposition is more rapid when they are stored in the dry form rather than as emulsions. The various nitrobenzoic acid esters of Vitamins D<sub>2</sub> and D<sub>3</sub> are more stable for they showed no apparent decomposition after being stored for five years.

When an emulsion of Vitamin D<sub>2</sub> in propylene glycol (drisdol) was diluted with water it lost as much as 75% of its potency during the period of bioassay, but when diluted with milk or propylene glycol it showed no deterioration.

Market milk fortified with Vitamin D<sub>2</sub> showed no apparent loss of potency when held for 8 days and evaporated milk, similarly fortified, showed no loss of Vitamin D after storage periods ranging from 6 to 15 months. A.O.C.

137. **Some Medical Aspects of Protein Foods.** FREDRICK J. STARE AND GEORGE W. THORN, Schools of Pub. Health and Medicine, Harvard Univ.; Med. Serv., Peter Bent Brigham Hosp., Boston, Mass. *Amer. Jour. Pub. Health*, 33, No. 12: 1444-1450. 1943.

The protein requirement of man cannot in the present state of knowledge be quantitatively stated in terms of amino acids, under certain standardized conditions. Calorie intake from carbohydrate and fat spares protein, and in the presence of sufficient calories from non-protein sources, the amount of protein in the diet of an active adult may be safely reduced to 50 grams per day, of which as little as 5 grams may be in the form of animal protein.

The protein requirement is not increased in exercise, and physical fitness and efficiency are not impaired or improved on low protein diets adequate in other nutrients. Substituting protein for some of the carbohydrate in the common American breakfast is a feasible way to prevent mid-morning symptoms of fatigue. High protein diets are not harmful to the normal adult and are of definite therapeutic value in many diseases and particularly in convalescence. Certain less common protein foods exist which are of high nutritional value, and which could be used with considerable value in post-war feeding operations. Protein foods are excellent sources of nutrients other than proteins, and we cannot reduce the protein of a diet unless at the same time we provide the other essential factors contributed by protein foods.

M.W.Y.

## MILK

138. **The Place of the Dairy Industry in Postwar America.** CHRIS L. CHRISTENSEN, Vice Pres., The Celotex Corp. *Milk Dealer*, 33, No. 4: 70-71. Jan., 1944.

Fluid milk production and distribution as a postwar product will experience many changes. Standardization of milk inspection laws and regulations; economies in transportation both from farm and to consumer and the possible inauguration of partial condensation by milk plants before delivery to consumers are postwar changes in the processing field. In the production field (1) improved feeding methods of both hay and grain by use of recommended feeding procedures to improve quality and nutrition of milk as well as quantity; (2) improved soil and soil cultivation methods; (3) use of commercial nitrogen fertilizers for increasing pasture and hay production; (4) herd improvement through use of artificial breeding; (5) improved dairy barns and housing facilities through better designing; and (6) general reduction of milking time and improved equipment will all be factors to consider as a postwar problem in the dairy industry. C.S.T.

139. **A Technical Survey of Commercial Cultured Skim Milk or Buttermilk Manufacture in the United States.** F. V. KOSIKOWSKY, Dept. of Dairy Indus., Col. of Agr., Cornell Univ., Ithaca, N. Y. *Milk Dealer*, 33, No. 4: 25-27, 44-46. Jan., 1944.

A cross-section survey of 45 dairy plants through the United States made by use of questionnaire was basis of this study. A range of from 3,650 to 12.5 million quarts with a total of 25.6 million quarts of cultured buttermilk annually was represented in the survey. Principal factors studied were (1) proportionate relationship of cultured buttermilk to whole milk sold ranging from 1.5 to 14.83% with average of 2.59%; (2) source of starter—88.9% commercial starter used; (3) amount of starter used ranging from



1.28 to 2.17% with average of 1.84%; (4) amount of added milk fat used ranging from 1.00 to 1.69% with average of 1.44%; (5) temperature and range of holding time for pasteurization, temperatures used ranging from 160 to 190° F. for 30 to 37 minutes; (6) temperature and time of incubation ranging from 65° to 75° F. and from 10 to 16 hours held; and (7) acidity of curd when broken, cooled, and stored, ranging from 0.67 to 0.85%. Other factors studied were addition of salt, gelatin, butter, and customer complaints. Wheying-off and viscosity were deemed greatest problems by the author. C.S.T.

**140. Addition of Citric Acid to Improve the Flavor of Cultured Butter-milk.** J. A. NELSON, Agr. Expt. Sta., Montana State College. Milk Dealer, 33, No. 4: 32. Jan., 1944.

The addition of citric acid to cultures used in producing cultured butter-milk, similar to use of citric acid in butter cultures, was studied in this experiment. Check sample of milk did not have citric acid added to cultures used but otherwise the samples were processed and treated similarly. The creatin test to determine quantitatively the acetylmethylcarbinol and diacetyl development showed in favor of citric acid cultures. Desirable flavor characteristics in all cultures to which citric acid was added were declared present by two competent culture judges. The author concludes "that the flavor of cultured buttermilk could be improved by the addition of citric acid to the culture milk before inoculation." C.S.T.

**141. Agreeable Manner Will Maintain Doorstep Delivery.** C. W. ES-MOND. Milk Plant Monthly, 32, No. 8: 29-30. 1943.

The author points out the importance of keeping the milk customer satisfied. Disgruntled milk customers do not really object to curtailment of deliveries; to the prompt return of milk bottles; to the prompt payment of milk bills; to the delivery of uniform quantities of milk daily so as to eliminate returns, or to other reasonable practices approved by good dairy management, but do object to the brusque, abrupt and blunt methods used by some milk men in putting these changes into effect. Since the customer is the ultimate boss not only of the milk men themselves, but of the dairy industry, it behooves the management to insist upon the milk men handling customers with "kid gloves." The customer will continue to prefer doorstep delivery if it is done at reasonable cost, and in an agreeable manner. Agreeable manner in doorstep delivery will not only insure the job of the milk man, but in the end will help to insure the job of all other milk men as well. G.M.T.

**142. Dye Reduction Tests for Heat Treated Milk.** A. L. PROVEN AND A. ROWLANDS. Dairy Indus., 8, No. 12: 693. Dec., 1943.

The authors do not recommend the colony count or the coliform test as

a means of controlling the keeping quality of heat treated milk. They suggest a half-hour methylene blue or resazurin test at 37° C. applied to samples which have been stored at 18° C. from 3 P.M. on the day of distribution until 10 A.M. the following day. This method can be relied upon to detect the majority of samples with an unsatisfactory keeping quality.

D.V.J.

143. **The Acidity Test Compared with the 10-Minute Resazurin Test and the Methylene Blue Test.** G. E. JONES AND H. BARKWORTH. Dairy Indus., 8, No. 11: 635. Nov., 1943.

A very significant correlation was found between the 10-minute resazurin test and the acidity of milk. The authors observed and presented data to support the fact that the 10-minute resazurin test is more closely affected by changes in acidity than is the methylene-blue test.

D.V.J.

144. **The Relative Keeping Qualities of Evening, Morning and Mixed Milk.** E. W. ERSKINE, B. M. FISCHER, S. M. L. SMITH, AND J. G. DAVIS. Dairy Indus., 8, No. 11: 618. Nov., 1943.

The authors found no marked differences in the bacteriological qualities of evening, morning and mixed milk. The methods used for this analysis were the "clot on boiling," methylene blue, resazurin, plate count and coli tests.

D.V.J.

145. **The Use of the Resazurin Comparator in Artificial Light.** J. G. DAVIS AND L. G. NEWLAND. Dairy Indus., 8, No. 10: 555. Oct., 1943.

In reading resazurin tests with the resazurin comparator daylight was found to be most suitable from all standpoints. However, low-pressure mercury vapor fluorescent tubular lamps gave very comparable results but were more fatiguing to the operator's eyes. In using this type of artificial light, it is desirable to exclude other extraneous light.

D.V.J.

146. **Thermoduric Organisms in Milk.** C. S. MORRIS AND M. EDWARDS. Dairy Indus., 8, No. 10: 550. Oct., 1943.

It is pointed out that heat-resistant types of organisms in milk can usually be traced back to a lack of sanitation on the farm or inefficient cleaning of milk cans at the creamery. Heat resistant coliform organisms may come from dried films on utensils or from contaminated water supplies.

Tests on raw milk are of little value in detecting thermoduric organisms. The author suggests that the pasteurized milk be examined by the following tests: 1. A phosphatase test immediately after pasteurization or when milk arrives at laboratory. Hold the milk for 24 hours at 18° C. and then run,

(a) a plate count at 30° C. or 37° C. on yeastrel milk agar (yeast extract milk agar), (b) a resazurin test at 37° C., and (c) a presumptive coliform test.  
D.V.J.

**147. The Resazurin Test for Sterility of Milk Cans.** J. G. DAVIS AND D. W. WATSON. *Dairy Indus.*, 8, No. 8: 415. Aug., 1943.

The authors attempted several procedures of holding rinse solutions prior to testing them by the standard resazurin technique. Plate counts were used as a basis of comparison. The most satisfactory procedure of those tried was the following: One ml. of rinse is inoculated into 10 ml. of separated milk and incubated at 22° C. in a water bath or large tank in a heated room for 24 hours. At this time a standard resazurin test is made at 37° C. Any sample which reduces to a disc number of 5 or less in 30 minutes is regarded as unsatisfactory and probably has a count of more than 500 per ml. of rinse.  
D.V.J.

**148. Cryophilic Bacteria in Relation to Milk Can Sterility Tests.** G. F. V. MORGAN. *Dairy Indus.*, 8, No. 8: 411. Aug., 1943.

The author found that the cryophilic organisms, isolated from milk cans after mechanical washing, were of a type usually associated with water. He points out that the detergent tank in the can washer frequently is the source of contamination and, therefore, should be cleaned after each day's operation. The rinse tanks on can washers are much less liable to become a problem. However, it is extremely important to keep the entire washer clean if contamination is to be avoided.  
D.V.J.

**149. Rapid Platform Tests.** H. BARKWORTH, J. G. DAVIS, J. W. EDGELL, A. ROWLANDS, AND D. W. WATSON. *Dairy Indus.*, 8, No. 5: 215. May, 1943.

In an attempt to find suitable tests for detecting unsatisfactory milks at the receiving platform, the authors investigated the following methods: smell and taste, clot on boiling, alizarin-alcohol, titratable acidity, pH, resazurin (10 minutes and 1 hour) and methylene blue. Although most of these tests had certain desirable characteristics, the 10-minute resazurin test was found to be most suitable for the purpose.  
D.V.J.

**150. Dye Reduction in Milk Related to Eh, pH and Dissolved Gases.** J. M. FRAYER. *Vt. Agr. Expt. Sta. Bul.* 498. Sept., 1942.

This publication contributes fundamental facts regarding the physiological processes involved in the application of the resazurin and the methylene blue tests for milk quality. The results as summarized are as follows:

"The reduction processes of the resazurin and methylene blue tests were

followed coordinately with Eh, pH and dissolved gas determinations. The results secured seem to warrant the following conclusions:

"1. There is little relationship between the moment of dye reduction and pH, oxygen depletion and pH changes not necessarily proceeding coincidentally.

"2. The bacterial growth phase at the moment of test initiation has much to do with the shape of the time: potential curve.

"3. The Eh level at the resazurin-pink stage of reduction is much more variable than it is when fully reduced.

"4. Most milks reduce resazurin to the pink stage at reduced oxygen levels, no case having as yet been observed of complete visual reduction of either dye in the presence of more than a minimum of oxygen.

"5. Milks held at 40° F. or less for 24 more hours tend to attain the resazurin-pink stage at higher Eh levels than when fresh.

"6. It can be safely assumed: (a) that all cell types found in milk have the same influence on dye reduction and gas depletion; (b) that oxygen is the only gas involved in the reaction; (c) that all unusual color modifications result from harmful abnormalities.

"7. The stage at which the carbon dioxide content is significantly increased is often accompanied by a rapid decline in both Eh and pH and by imminent color reduction.

"8. A low count milk sample to which resazurin is added, when exposed to bright sunlight and unaccompanied by metabolic activity previous to incubation, behaves as a rule as does a sample of poor milk in respect to the rapidity of oxygen depletion, of negative Eh swing and of color fading.

"Because of the relatively high sensitivity of the resazurin dye and the possible occurrence of color changes resulting from variations in factors other than those attributable to a high bacterial content and/or to physiological or pathological abnormalities, the opinion is advanced that, in the absence of microscopic confirmation, the methylene blue test for milk quality is likely to afford better results in the hands of the average milk plant operator than are any of the known modifications of the resazurin test."

P.H.T.

**151. Mold Growth in Composite Milk Samples.** J. M. FRAYER, Vt. Agr. Expt. Sta., Burlington, Vt. Pamphlet No. 2. April, 1943.

Mold growth in composite milk samples is objectionable because certain types of mold elaborate enzymes which may hydrolyze fat and consequently may lower the test as much as 0.3 to 0.4%. Mold growth also interferes with adequate sample mixing and pipetting.

To control this problem the mold must be prevented from getting into the sample. This can be done by using clean methods in handling the samples, by thoroughly cleansing all sample bottles, stoppers and storage boxes be-

tween each sampling period, and by adequate germicidal treatment of sample bottles and stoppers.

P.H.T.

152. **Bi-Monthly or Monthly Testing at Milk Plants.** D. W. WHITMAN, R. O. SLACK, AND E. O. HERREID, Vt. Agr. Expt. Sta., Burlington, Vt. Vt. Agr. Expt. Sta. Bul. 502. June, 1943.

As a labor-saving measure, composite samples may be tested monthly instead of bimonthly provided the bottles are kept tightly stoppered and are stored at 50° F. or below while not in use. It is also important to keep the samples free from mold growth and to properly prepare the samples for testing. Tests conducted in a commercial milk plant supplied by 113 patrons showed that the bi-monthly composites yielded only slightly more fat than the monthly composites. The preserved composite samples were prepared for testing by warming to 95–100° F. in a water bath at 107–110° F. Any adherent cream was carefully brushed loose. The samples were mixed by pouring four times. After pipetting, the warmed milk was permitted to cool to 70° F. before 15 ml. of sulfuric acid at 70° F. was added to the sample. Mixing of the acid and milk was done in a mechanical shaking device. The tests were read to 0.025% but were recorded to the nearest 0.1%.

P.H.T.

## PHYSIOLOGY

153. **The Effects of Mild Hyperthyroidism on Growing Animals of Four Species.** M. KOGER AND C. W. TURNER. Mo. Agr. Expt. Sta. Res. Bul. 377. 75 pages. Sept., 1943.

Growing animals of four species, including mice, rats, guinea pigs, and rabbits, were treated with thyroactive preparations varying in amounts from relatively large dosages which were toxic to very small dosage which apparently did not affect growth or were so small in amount that it appeared impractical to attempt further reduction in dosage.

The growth rate of mice was consistently and significantly increased by treatment with a rather wide range in dosage (0.01 to 0.04 mg. thyroxine-sodium daily or 0.04 to 0.32 per cent thyroactive iodocasein in the ration while maximum size attained by control and treated animals was unchanged. Feed intake of mice was increased by treatment. The treated animals stored more protein and more body weight per unit of feed consumed than controls while control animals were more efficient in storage of fat and energy.

The effect of feeding thyroactive iodocasein to rats was variable with strain and sex. There was some evidence of slight acceleration of growth in weight of a few females due to feeding thyroactive iodocasein, but for the most part body weight was unaffected or depressed. The nose-anus length

of male rats of one strain (Sprague-Dawley) was increased due to treatment, but was not observed in any of the other animals. Male rats were less tolerant of thyroactive preparations than female.

The growth rate of male guinea pigs was slightly accelerated by mild treatment (0.0025 to 0.0075 per cent thyroactive casein in the ration) for a short period of time, but the same treatment later became toxic with increase in age and arrival of warm weather. Growth rate of female guinea pigs was not affected for a few weeks, after which time treated animals ceased growing and lost weight.

Small amounts of thyroactive casein (0.0025 to 0.02 per cent of the ration) apparently did not affect growth of rabbits while larger amounts caused a depression of growth.

The effect of thyroactive casein on the organ weight of rats was studied. Extremely small dosages given to males did not affect the weight of any of the glands or organs weighed, although the thyroids of treated animals showed histological evidence of inactivity. Larger dosages given to either sex resulted in hypertrophy of heart, liver and kidneys. The effects on other glands was variable with strain and sex of animals.

The thyrotropic potency of the pituitaries of animals was markedly lowered by feeding thyroactive iodocasein.

15 figures, 26 tables, 7 pages of literature references.

J.G.A.

**154. The Mammogenic Hormones of the Anterior Pituitary. II. The Lobule-alveolar Growth Factor. J. P. MIXNER AND C. W. TURNER. Mo. Agr. Expt. Sta. Res. Bul. 378. 62 pages, illus. Sept., 1943.**

1. Mammary lobule-alveolar growth responses were secured in castrate virgin female mice with anterior pituitary materials injected over periods of time ranging from four to ten days. These responses were not very predictable or repeatable on reassay.

2. The simultaneous injection of pituitary preparations and a small amount of estrone greatly reduced the amount of pituitary required to secure alveolar responses and the dosages of pituitary preparations injected were proportional to the per cent positive lobule-alveolar responses secured in groups of assay mice.

3. In the development of an assay method for the mammogenic lobule-alveolar growth factor, a ten-day assay period was found to be optimal.

4. The length of time elapsing between ovariectomy of the assay mice and the beginning of injection affects the mammary response secured; the shorter the time, the greater the mammary response.

5. An assay for the mammogenic lobule-alveolar growth factor was formulated. A mouse unit of this factor was defined as the amount of material required per mouse injected over a ten-day period to obtain minimal lobule-alveolar growth in  $50 \pm 10$  per cent of ten or more castrate nulliparous

female mice when a total of 75 I.U. of estrone is simultaneously injected. Injection of the assay animals should start immediately after ovariectomy unless they are first primed with estrogen for several days preliminary to the start of injections.

6. Estrone in amounts of 40 I.U. to 133 I.U. were found to synergize best with one mg. of progesterone in stimulating mammary lobule-alveolar growth. Greater or smaller amounts of estrone did not give optimum synergism with progesterone.

7. Progesterone or pregnenolone alone caused lobule-alveolar growth. However, five or six times as much was required as if estrogen was also injected.

8. Although 2400 I.U. of estrone was able to completely inhibit the activity of a mouse unit of progesterone (one mg.), it was unable to inhibit the activity of a mouse unit of a pituitary preparation.

9. As a result of a series of experiments with progesterone, pregnenolone, pituitary extracts and estrogen, it was suggested that estrogen enhances the activity of progesterone and pituitary materials in stimulating mammary lobule-alveolar growth by acting directly on the stromal tissue surrounding the mammary gland producing an increased hyperemia and vascularity associated with an increased permeability of the vascular system. This condition would allow a circulating pituitary mammogen to be maximally effective in causing mammary gland growth.

10. Both estradiol benzoate and diethylstilbestrol were able to substitute for estrone in conjunction with progesterone in enhancing mammary lobule-alveolar growth.

11. Assays of various types of pituitary extracts showed that the mammogenic lobule-alveolar growth factor is protein in nature. These assays also indicate that this factor is not identical with lactogen, thyrotropin or gonadotropin.

12. Progesterone, pregnenolone, desoxycorticosterone, dehydroandosterone, diethylstilbestrol, acetoxy-pregnenolone, and methyl testosterone ranked in the above order in their ability to stimulate mammary lobule-alveolar growth.

13. High environmental temperature inhibited the ability of progesterone and estrone to stimulate mammary lobule-alveolar growth. This same high temperature was unable to inhibit the ability of a pituitary preparation to stimulate lobule-alveolar growth.

14. Thyroxine in suitable amounts increased by about 33% the efficiency of progesterone in stimulating lobule-alveolar growth.

15. Thyroidectomy greatly decreased the efficiency of both progesterone and pituitary preparations in stimulating mammary lobule-alveolar growth.

16. Virgin female goats injected daily with twenty or thirty mg. of progesterone plus 100 or 150 micrograms of diethylstilbestrol, respectively,

for sixty days were stimulated to develop mammary glands similar to that seen in midpregnancy. Twelve days additional treatment with 0.25 mg. daily of diethylstilbestrol caused an initiation of secretion in these mammary glands similar to that seen at the time of parturition.

17. The response of virgin female goats to diethylstilbestrol in regard to mammary lobule-alveolar growth was extremely variable. In some cases very slight stimulation of the lobule-alveolar system was effected while in others a considerable development was secured.

18. Lobule-alveolar growth secured with diethylstilbestrol injections in goats was not histologically typical of that seen in normal lactating glands. The alveoli were much larger and less dense than normal lactating alveoli. Abnormal papillae-like structures were seen protruding into the lumina of the alveoli.

19. The over-all picture of mammary gland development as affected by the various endocrine glands was discussed.

20 figures, 18 tables, 6 pages of literature references.

J.G.A.

**155. The Effect of Thyroxine and Dinitrophenol on Sperm Metabolism.**

HENRY A. LARDY AND PAUL H. PHILLIPS, Dept. Biochem., Univ. Wis. Jour. Biol. Chem., 149, No. 1: 177. 1943.

Dinitrophenol, a substance which stimulates tissue respiration, stimulated both the glycolysis and respiration, in the presence of metabolites, of bull spermatozoa, but it inhibited endogenous respiration. Dinitrophenol inhibited sperm motility and this could be prevented, to some extent, by the addition of glucose, lactate, or pyruvate.

"Thyroxine in 1:75,000 dilution inhibited respiration of bull spermatozoa and stimulated glycolysis. Orthothyroxine, an isomer of low physiological activity, did not significantly affect either glycolysis or respiration."

A.O.C.

## MISCELLANEOUS

**156. Reducing Hazards of Winter Truck Operation.** E. G. QUESNEL, Dir. of Safety, The Borden Co. Milk Dealer, 33, No. 4: 24. Jan., 1944.

The protection of life and the prevention of accidents, particularly under extra hazards of winter driving when streets are icy, snowy, and full of ruts, is of first importance in the operation of milk delivery trucks. The driver is admonished to distribute load evenly, to keep windshield clear, avoid use of brakes, keep out of ruts, shift gears on hills, brake with engine, drive slowly and carefully and check motor combustion and exhaust. Extra precautions should be taken in meeting, following, and passing other cars and trucks.

C.S.T.



# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

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### MILK AND MILK PRODUCTS

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## JOURNALS

American Butter Review	Journal of Bacteriology
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American Journal of Diseases of Children	Journal of Dairy Research
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## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	Prussian Dairy Research Institute, Kiel, Germany
International Association of Ice Cream Manufacturers	State Agricultural Colleges and Experiment Stations
International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### BOOK REVIEW

157. **Handbook for the Etiology, Diagnosis and Control of Infectious Bovine Mastitis.** IVAL ARTHUR MERCHANT AND R. ALLEN PACKER, Iowa State College. Published by Burgess Publishing Co., Minneapolis, Minn. 66 pages. \$1.25.

The authors of this handbook should be commended for their effort to gather scattered information regarding an important animal disease, sift out a great deal of cumbersome detail and concentrate the important factual matter between the covers of a small handbook.

This work will be a useful addition to the libraries of teachers, students and practitioners. Predisposing influences, bacteriology, diagnosis, control and treatment of the disease are the important subjects discussed. Sufficient detail is included to make the work a useful guide in procedures and techniques.

T.S.S.

### BACTERIOLOGY

158. **Effect of Increase in Acidity on Antiseptic Efficiency.** OTTO RAHN AND JEAN E. CONN, N. Y. State Col. of Agr., Cornell Univ., Ithaca, N. Y. Jour. Indus. and Engin. Chem., Indus. Ed., 36, No. 2: 185. Feb., 1944.

Benzoic acid, salicylic acid, and sulfurous acid are nearly a hundred times as efficient antiseptics in strongly acid solutions as they are in neutral solutions. The toxic principle of benzoic and salicylic acids is the undissociated acid molecule. Growth of a wine yeast was completely suppressed when the concentration of undissociated benzoic acid reached 25 mg. per 100 ml. or when 4 mg. of undissociated salicylic acid was present. When sulfur dioxide dissociates in water the  $\text{HSO}_3^-$  ions inhibit the multiplication of *B. coli* but not of yeast. The rapid death of yeast is brought about by 7 to 8 mg. of undissociated  $\text{H}_2\text{SO}_3$  per 100 ml.; *B. coli* can tolerate nearly ten times as much.

B.H.W.

### BUTTER

159. **Sanitation in Buttermaking.** WENDELL VINCENT. Amer. Butter Rev., 5, No. 8: 238-241. 1943.

The inspector and plant operator are responsible for practices affecting sanitation within the plant as well as with the finished product. Sediment testing of cream is necessary and straining of cream by means of moving cloths rather than by in-line filters should be advised. Use of polluted water

and regard of pasteurization as a cover for poor sanitation are common offenses. Neglect in making repairs by soldering, improper care of stuffing boxes, poor cleansing and rinsing, and insanitary handling of butter scraps result in the production of lower score butter. Cream shipped by cream stations and independent buyers is of much lower quality than that shipped by direct shippers in the ratio of 2:1. While producers have been urged to increase quantity no one has ever urged that this be done at the expense of quality.

P.S.L.

160. **Butter Outlook and Its Relationship to Milk Solids for Ice Cream for 1944.** T. G. STUTTS, Chief, Dairy and Poultry Branch, F.D.A., Washington, D. C. *Ice Cream Trade Jour.*, 40, No. 1: 12. Jan., 1944.

A comprehensive picture is drawn by the author of the government's effort to apportion the available milk supply to production of the various dairy products required for civilian, military and lease-lend purposes. Ice cream manufacture is not likely to be further restricted inasmuch as the dairy industry will of necessity "lean heavily" on the ice cream industry to absorb milk solids when the food emergency is over. Butter is likely to be short for the duration but supplies should not become much scarcer than at present. If milk production does not "fall off" too much and if conservation order, F D O 79, works as it should, no coupon rationing of fluid milk will become necessary. A huge amount of cream has been moving into cold storage which tends to dislocate the dairy products program. Much of this should go into butter but it is doubtful if it was stored for that purpose. On the whole, the dairy products program seems to be working quite satisfactorily.

F.J.D.

## CHEESE

161. **A Program for Maintaining Cheese Quality.** G. H. WILSTER, Oreg. State Col., Corvallis. *Natl. Butter and Cheese Jour.*, 35, No. 2: 16. Feb., 1944.

The program recommends: grading milk by appearance, sediment test, flavor, methylene blue and curd tests; washing, sterilizing and drying cans; pasteurization of milk; the use of an active starter; limitation of acid development during manufacture to conform to a making period of about 5 hours from setting to milling; strict sanitation in factory maintenance; and careful curing at temperatures selected to bring out the best flavors.

W.V.P.

162. **Did You Ever See a Dream Working?** PAUL MANDT, Olsen Pub. Co., Milwaukee, Wis. *Natl. Butter and Cheese Jour.*, 35, No. 2: 8. Feb., 1944.

A practical version of an ideal cheese factory is described and discussed. This well-equipped plant includes such unusual machinery as an autoclave

for starter cultures, a mechanically refrigerated starter can and glass holding tubes combined with a plate-type, regenerative pasteurizer. Cleanliness is emphasized in plant maintenance and is accomplished by systematic routines, simple instructions and assignment of responsibility to individuals. The plant operates with dry floors during the day. Making operations are run "by the clock" because of starter control methods and pasteurizing efficiency.  
W.V.P.

163. **Some Causes of Slow Production of Acid During Cheesemaking.** G. H. WILSTER, *Oreg. State Col., Corvallis. Natl. Butter and Cheese Jour.*, 35, No. 3: 10. March, 1944.

The common trouble of a starter "going dead" may be caused by bacteriophage, an unidentified agent which "eats" starter bacteria. Phage may be found in sewage, faeces, pus, and the dust and whey in a cheese factory. It gets into starter by contamination with mist from the whey separator, factory air and dust. The infection may last for months in a factory. Phage is destroyed by treatments which destroy the organisms which it attacks. Prevention of phage infection may be accomplished by disinfecting the factory by spraying, by protection of starter cultures from air-borne phage and by preparation of starter in a separate building designed to exclude phage-laden air.  
W.V.P.

164. **A Program for Maintaining Cheese Quality.** G. H. WILSTER, *Oregon State Col., Corvallis, Oreg. Amer. Butter Rev.*, 5, No. 9: 270-272, 283-284. 1943.

In view of the precarious situation in the cheese industry due to the manufacture in many cases of a low quality of product the author has outlined and discussed rather completely nine factors important in the improvement of cheese. These are: 1, the grading of milk and rejection of poor quality product; 2, thorough cleansing and proper care of cans after washing; 3, efficient pasteurization of milk; 4, proper use of a good starter; 5, unhurried methods of manufacture with especial attention given to acid development; 6, sanitation; 7, curing at low temperatures and moderate humidity; 8, regulation of moisture and fat content through adequate and regular laboratory control; and, 9, grading as a means of improvement.  
P.S.L.

## CHEMISTRY

165. **The Total Nitrogen Content of Egg Albumin and Other Proteins.** A. C. CHIBNALL, M. W. REES, AND E. F. WILLIAMS, *Biochem. Dept., Imperial Col., London, S.W. 7. Biochem. Jour.*, 37, No. 3: 354-359. Sept., 1943.

With the development of new catalysts for the Kjeldahl nitrogen determination, and especially with the advent of the micro Kjeldahl method, there

has been a tendency to reduce the time required for digesting the sample. The authors feel that this has been done at the expense of accuracy, for the rapid digestion gives lower percentages of nitrogen than those reported by such earlier workers as Osborne and Campbell, as well as their own results with longer digestion periods. "With proteins and protein hydrolysates it is necessary to continue the heating for 8 hours or more after the digest has cleared." In reporting nitrogen determinations on proteins there should be more details of the procedure given, as well as some history of the treatment of the protein or its method of preparation. This may help to intelligently interpret the results.

For casein, prepared by the method of Cohn and Hendry they report 15.73% nitrogen on a moisture-free and ash-free basis. For B-lactoglobulin, which was twice recrystallized, coagulated by heat and washed free from inorganic salts and dried, they report a value of 15.58% nitrogen on a moisture-free and ash-free basis.

Abstractor's note: The term B-lactoglobulin is not frequently used in literature in America. There is a need for a unified system of nomenclature in the field of the milk albumins and globulins. The B-lactoglobulin referred to above is apparently that fraction of the milk albumin which was crystallized by A. D. Palmer. This is the only milk protein reported to have been crystallized to date.

A.O.C.

166. **The Dicarboxylic and Basic Amino Acids of Edestin, Egg Albumin and B-Lactoglobulin.** A. C. CHIBNALL, M. W. REES, AND E. F. WILLIAMS, Biochem. Dept., Imperial Col., London, S.W. 7. Biochem. Jour., 37, No. 3: 372-388. Sept., 1943.

A detailed procedure for the estimation of dicarboxylic and basic amino acids is given. The method is admittedly a long one, requiring about 900 working hours, but the authors state that the results are more reliable than those of any method reported heretofore, accounting for all but 1.25% of the total protein nitrogen. The method differs from those usually used in that no reagent is added to the hydrolysate unless it can be quantitatively removed later without carrying with it an appreciable amount of protein.

For B-lactoglobulin the percentages of the total nitrogen as the dicarboxylic acids glutamic and aspartic are given as 13.14% and 6.68% respectively, while the basic amino acids are: arginine 5.95% histidine 2.69% and lysine 12.07% of the total nitrogen.

A.O.C.

167. **Oxidative Rancidity in Edible Fats.** L. R. BRYANT, Ont. Agr. Col., Guelph, Ontario. Food in Canada, 4, No. 1: 7. 1944.

Atmospheric oxygen produces a type of rancidity in food fats characterized by changes in color, destruction of the fat-soluble vitamins and the development of off-flavors. The chemical make up determines the suscepti-

bility of various fats to this chemical change. Aside from the fat itself, such factors as temperature, light, ozone, metals or the presence of anti-oxidants accelerate or inhibit the reaction rate. Tests for the susceptibility to, and degree of, oxidation are described. O.R.I.

168. **The Causes, Cures and Methods of Preventing Rancidity.** C. H. CASTELL, Ont. Agr. Col., Guelph, Ontario. Food in Canada, 3, No. 10: 11; 3, No. 11: 11; 3, No. 12: 10. 1943.

This series of three articles deals with fat spoilage from the standpoint of (1) hydrolytic rancidity of non-microbial origin, (2) rancidity and off-flavors produced by yeasts, molds and bacteria of the aerobic group, and (3) off-flavors of a similar character produced by the butyric-acid-forming anaerobes. In the first of these, the chemical make-up of fats is described with stress being placed on strong odors and flavors possessed by some of the free fatty acids. The sources and characteristics of the lipases are discussed and methods of measuring rancidity and the lipase content of foods described. Particular attention is given to milk and dairy products.

In the second article dealing with microbial agencies causing food spoilage it is pointed out that oxidative rancidity is often an important secondary reaction occurring after the fat has first been attacked by bacteria. Yeasts are very rarely lipolytic but most molds produce lipase. Fat-splitting bacteria are numerous and widely distributed and many species grow at low temperatures. Members of the *Alcaligines*, *Aerobacter*, *Achromobacter*, *Pseudomonas* and *Serratia* genera are of greatest importance. Some of the newer methods whereby organisms of this group may be identified or counted are described.

Butyric-acid-forming anaerobes produce a type of rancidity in some foods entirely unrelated to the fat content. These organisms are strict anaerobes, and produce heat-resistant spores. Butyric acid is one of the chief products produced when carbohydrates are fermented. Relatively few species grow in an acid medium or at low temperatures. Culturing and counting require the use of either an anaerobic jar or growth in a corn-liver or cereal grass medium. Dairy products, particularly some European varieties of cheese, sometimes develop rancid flavors as a result of the growth of organisms of this group. O.R.I.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

169. **Improving Keeping Quality of Dry Whole Milk.** C. D. DAHLE AND D. V. JOSEPHSON, Dairy Dept., Penn. State Col. Milk Plant Monthly, 32, No. 10: 28-29. 1943.

By removal of a large percentage of lecithin through churning and separating out the butter oil and through supercentrifuging the skimmilk,

atmospheric roll dry whole milk of good keeping quality could be made from the recombined product. The possibility of removing some other pro-oxidant than lecithin by the supercentrifuging treatment was suggested by the great improvement in keeping quality noted. The prevalent flavor defect occurring in dry whole milk is of an oxidative type. G.M.T.

## FOOD VALUE OF DAIRY PRODUCTS

170. **Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure.** PAUL D. BOYER, ROBERT SPITZER, CURTIS JENSEN, AND PAUL H. PHILLIPS, College of Agr., Univ. of Wis., Madison, Wis. *Jour. Indus. and Engin. Chem., Analyt. Ed.*, 16, No. 2: 101. Feb., 1944.

The authors describe a rapid procedure for the extraction and determination of vitamin A and carotene in milk. Two volumes of milk mixed with 3 volumes of alcoholic potassium hydroxide are allowed to stand for 3 hours at room temperature. The mixture is then extracted twice with ether and the vitamin A and carotene are determined by means of the Carr-Price reaction and with the aid of an Evelyn photoelectric colorimeter. The new procedure was compared to the longer procedure of Olsen, Hegsted, and Peterson. The comparative analyses showed that with a single ether extraction the new method occasionally gave low results for vitamin A. The double extraction procedure which was described gave good checks with both pasteurized and raw milks from cows of various breeds. The carotene determination as given in the procedure is a measure of the total carotenoids in the milk. B.H.W.

171. **Determination of Vitamin A and Carotenoids in Butterfat. Comparison of Direct Spectrophotometry with Filter Photometry and Use of the Antimony Trichloride Reaction.** F. P. ZCHEILE, H. A. NASH, R. L. HENRY, AND L. F. GREEN, Purdue Univ. Agr. Expt. Sta., Lafayette, Ind. *Jour. Indus. and Engin. Chem., Analyt. Ed.*, 16, No. 2: 83. Feb., 1944.

The data reported were obtained during a comparative study of methods for the determination of vitamin A and carotenoids in butter by the Technical Committee on Vitamin A Researches in cooperation with the National Cooperative Project on the Vitamin A Potency of Market Butters. Six representative samples of butterfat from sweet cream were prepared and analyzed by seven collaborators. This paper compares the results of the direct spectroscopic method used by the authors and the colorimetry methods used by the other collaborators. Comparison of the carotenoid content of the butterfats determined by the different methods showed that from the total of 42 determinations, only 5 deviated from their corresponding means



by more than 7%, the maximum deviation being 13.3%. The direct spectrophotometry results had both the smallest mean absolute deviation and the smallest maximum deviation. Comparison of the determinations of vitamin A contents showed the results from direct spectrophotometry to have mean absolute and maximum deviations two-thirds as great as the over-all averages. The antimony trichloride reaction was considered the preferred physico-chemical method available for butters containing azo dyes.

B.H.W.

172. **Some Experiments on the Possible Relationship between Vitamin C and Calcification.** GEOFFREY H. BOURNE, Univ. Lab. of Physiol., Oxford. *Jour. Physiol.*, 102, No. 3: 319. Dec., 1943.

"Bone salt does not appear to be deposited (except in severe scurvy) until there is an adequate fibrous matrix to receive it. The production of the fibrous matrix of bone and the deposition of bone salt are therefore simultaneous processes. It would seem that as long as there is sufficient vitamin C to produce matrix then that matrix will be calcified. The function of vitamin C in bone formation appears to be to facilitate the production, not just of bone matrix, but of bone matrix impregnated with phosphatase. There is no evidence that vitamin C can be regarded as a coenzyme of phosphatase in calcificatory processes. The apparent reduction of phosphatase activity in scurvy is therefore probably due to a reduction in the amount of bone matrix produced.

"That it is actually vitamin C and not some associated impurity that is responsible for this is suggested by the fact that the administration of vitamin P (citrin) and sodium citrate did not result in the formation of more osteoid trabeculae or the deposition of more bone salt than vitamin C alone."

D.E.

## HERD MANAGEMENT

173. **Preparing the Cow for Milking.** W. E. PETERSEN, Prof. of Dairy Husbandry, Univ. of Minn. *Milk Plant Monthly*, 32, No. 12: 26-27. 1943.

"Letting down" of milk is the result of a positive act causing tiny muscle cells to contract thus squeezing the milk out of the alveoli where it is made. This is a reflex act, spontaneous to a stimulus, which causes the pituitary gland to secrete a hormone (oxytocin) into the blood by which it is carried to the mammary gland.

The following fundamentals about the response and action of this reflex furnish the basis for several recommended practices: 1. Response to the stimulus is interfered with by any condition that distracts the cow. 2. Once the "let down" has occurred, it is effective for but a short period of time.

3. Cows may become conditioned to let down their milk to a number of different stimuli. 4. The way a cow responds to milking is determined largely by training. 5. About 45 seconds are required from the application of the stimulus to the "let down" of the milk.

The following 11 rules are based on these fundamentals: 1. Handle heifers carefully when they are first milked. 2. Avoid the unusual during milking. 3. Do not treat the cow roughly at any time. 4. The milker must be a person who does not arouse the cow's suspicion. 5. Do not wash or massage the udders or stimulate cows in other ways to let down their milk before milking is to begin. 6. Milk those cows first which let down their milk in response to preparations for milking. 7. Milk rapidly. 8. Do not practice prolonged stripping. 9. Operate milking machines according to the manufacturers' directions. 10. Do not leave the milking machine on the cow after the milk has ceased flowing. 11. Develop the technique of knowing by feel when the gland has been emptied of milk.

For the best operation of milking machine the following 4 points must be observed: 1. Stimulate the cow to let down her milk about one minute before attaching the machine. 2. Operate the machine according to the manufacturer's directions. 3. As soon as the teat cups begin crawling upward begin stripping by tugging downward with sufficient force to lower the teat cups part way down on the teat and at the same time stroke each quarter downward in succession with the free hand. 4. Remove the machine as soon as milk ceases flowing.

G.M.T.

## ICE CREAM

174. **Ice Cream with the A.E.F.** S/Sgt. J. A. RABUFFO, E.T.O., England. *Ice Cream Trade Jour.*, 40, No. 2: 24. Feb., 1944.

After mentioning the shipment of ice cream freezers, fountains, ice cream mix and accessories which have been authorized for distribution and installation in the European Theater of Operations to supply Uncle Sam's soldiers with fountain products which they have greatly missed, "G. I. Joe" tells of the ingenuity of the cooks and "K.P.'s" in improvising ice cream freezers and ice cream mixes under all manner of circumstances. He admits that results rarely compare favorably with the ice cream to which they have been accustomed but are far better than none. In many cases arrangements have been made with local ice cream makers (who no longer make) to use freezing equipment, if the commissaries are able to provide some form of mix ingredients. "G. I. Joe" presents one cook's closely guarded secret formula, revealed in a weak moment, as being composed of: evaporated milk, powdered milk, water, sugar, powdered eggs, corn starch and a little salt. The concoction is brought to a boil, cooled and frozen.

F.J.D.

175. **Pooling Cabinet Service.** W. H. SNEATH, William Neilson, Ltd., Toronto, Canada. *Ice Cream Trade Jour.*, 40, No. 2: 30. Feb., 1944.

A pooled service for checking, adjusting and repairing refrigerated cabinets belonging to Canadian ice cream manufacturers is explained. The system was applied throughout the country by establishing zones, and all prices for parts, mileage rates, hourly wages, etc., were made uniform for each zone. One company was made responsible for all services in each zone and while the yearly records have not been compiled and summarized, there is little question but what tremendous savings have been achieved in gasoline, tires, mobile equipment, manpower and in the avoidance of breakdown. It is emphasized that successful operation of such a cooperative service can only be obtained through the confidence and cooperation of the entire personnel as well as the organizations involved. F.J.D.

176. **Sherbets—What About Their Future?** V. M. RABUFFO, *Ice Cream Trade Jour.*, New York City. *Ice Cream Trade Jour.*, 40, No. 2: 16. Feb., 1944.

With an estimated production of 75,000,000 gallons of sherbets and ices forecast for 1944, the author reviews the experiences of the ice cream industry during 1943, pointing out the errors made in "forcing" these products on dealers as a result of FDO-8 rather than preparing the way by a "selling" campaign. Opinions and experiences are cited and the potentialities of sherbets and ices in the post war picture are stressed. It is pointed out that much progress has lately been made in improving the quality of sherbets and ices and since there is a strong possibility of continued rationing of milk solids, for a period of time after the war, it is suggested that the industry should attempt to correct its recent error by engaging in an extensive effort to sell consumers on the "goodness" and palatability of these milk-solids-sparing products. Sherbets and ices play too vital a role and represent too large a volume to be neglected. The situation is a challenge to the ice cream industry. F.J.D.

177. **The Bacterial Content of Shell Eggs.** C. K. JOHNS, Bact. and Dairy Research, Dom. Dept. of Agr., Ottawa, Canada. *Food in Canada*, 3, No. 12: 15. 1943.

Bacteria count limits were incorporated into the 1943 Canada-Great Britain dried egg contracts. To learn whether or not shell eggs contribute materially to the count of the powder, bacterial numbers were determined on whole eggs by smearing 1/2000 g. loopfuls of mixed egg on tryptone glucose extract milk agar slants. Ninety per cent of the 348 eggs examined gave counts under 10,000 per g. with only one egg in excess of 500,000 per g. The eggs were all 2 months old when examined. O.R.I.

178. **Concrete in Ice Cream Plants.** LAWRENCE FLYNN. Ice Cream Field, 43, No. 1. Jan., 1944.

Floors which give the best service in food plants must have a top surface which wears well and resists the attacks of fruit juices, sugar solutions, and lactic acid, the author states.

Floors with porous surfaces are much more readily affected by acids and sugar solutions than are those with impervious surfaces. Special treatments can be employed to make porous surfaces practically impervious.

Dairy and ice cream plant floors are subject to hard usage and should be heavy duty concrete floors. The following basic principles are listed as essential in producing wear-resistant floor finishes: 1. Use only suitable, clean materials. 2. Use not in excess of 4½–5 gallons of mixing water per sack of cement. 3. Avoid "segregation" resulting in free water and fine material on top surface. 4. Keep concrete damp as long as possible. At least a week is required for normal Portland cement and three days when high early Portland cement is employed.

The importance of selecting suitable aggregate material and the use of proper proportions of ingredients as well as employing the proper methods and procedures are stressed. To avoid cracking of floor surfaces the author suggests the use of light wire mesh, 4 × 4 inch, No. 10 gauge wire weighing 31 pounds per 100 sq. ft. near the middle of the top or wearing course, and to avoid severe wear it is considered important to have as much coarse aggregate as possible near the surface.

Two methods are described for treating floor surfaces in order to make them impervious. One treatment consists in the application of warm linseed oil, Chinawood oil, or soy bean oil. To assist penetration the first coat should be thin (equal parts of oil and turpentine or other thinner). A second application can be given with a greater proportion of oil to thinner after the first coat has dried. A second method is the application of paraffin. It should have a melting point of 150° F. and should be applied as a paste prepared by melting 4 parts paraffin with 1 part turpentine and 16 parts toluol. Apply with brush and allow to penetrate 24 hours, keeping the floor warm; then polish with polishing machine.

Directions are also given for re-surfacing old floors after chipping away the old concrete to a depth of 1 inch.

W.C.C.

## MILK

179. **Influence of Temperature in Open and Closed Truck Hauling.** C. M. PESCK, Dairy and Food Dept., Minneapolis, Minn. Amer. Butter Rev., 5, No. 9: 274–276. 1944.

Roof temperature under the same conditions was 16° lower on a cab made from aluminum-painted wood than on black iron. During each stop

roof temperature increased 6°. Cans of cold water and cream appreciably affected the temperature in the truck, lowering it to 70° when the outside temperature at the start was 92°, and 97° at the end of the trip.

With an open truck and cans covered with a canvas the temperature on the floor of the truck was 85° when the cans were first loaded and 88° on arrival at the creamery. Outside temperature was 86° at the start of the trip, 94° at the end. Temperature varied with the wind direction and did not steadily decrease as in the closed truck. Acidity of the cream sharply increased in the canvas-covered open truck. In the original article an accompanying table gives detailed results. P.S.L.

180. **Testing Homogenized Milk.** S. T. COULTER, Univ. of Minn., Minneapolis, Minn. *Amer. Milk Rev.*, 5, No. 12: 382. 1943.

The method previously proposed by J. C. Marquardt of the New York (Geneva) Agricultural Experiment Station was given several trials at the Minnesota Dairy Department and gave fairly satisfactory results. The method developed is one of modifying the ordinary Babcock procedure. P.S.L.

181. **Bad Flavors in Milk.** E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y. *Amer. Milk Rev.*, 5, Nos. 11 and 12: 338-346, and 372-385. 1943.

This article combines in an interesting and helpful manner the experimental work of many writers as regards flavors that may occur in milk, together with a discussion of conditions favoring their presence and controlling the degree to which they are present. Those discussed more fully are feed, rancid, lipolytic, those due to fat content and to treatment of milk, as homogenization, oxidized, metallic, cooked, disinfectant, and absorbed flavors. P.S.L.

182. **Milk—Dairy Products Problems.** LELAND SPENCER, Cornell Univ., Ithaca, N. Y. *Amer. Milk Rev.*, 5, No. 9: 276-282. 1943.

More milk has been produced yearly than on the average for 1935-9 but demand has been much greater than production. The military has taken 19% of the production ordinarily available. As compared with 1935-9, consumption by civilians was lower in 1942 by 7% but up 10% for fluid milk, 13% for canned milk, 14% for cheese, and 67% for ice cream. Besides these demands there are those of the export market. Price ceilings and shortage of labor and feed have added to production difficulties. Price regulations have caused in many cases price maladjustments that become disruptive. To cushion to as great extent as possible the effects of the probable shortage this fall the author suggests six remedies: 1, adjustment of price ceilings in markets operating at a disadvantage; 2, confine shipment of milk

within 500 mile distances; 3, grant priority to milk within its own milk shed; 4, ban all sales of cream if necessary; 5, limit fluid milk sales if necessary; 6, ration milk but only if absolutely necessary and as a last resort. P.S.L.

183. **A Portable Resazurin Outfit.** J. G. DAVIS, Natl. Inst. for Res. in Dairying, Shinfield, Reading. *The Milk Indus.*, 24, No. 6: 47. Dec., 1943.

A portable test kit has been developed for the application of the resazurin test in the field. The outfit is essentially designed for use on the farm and in small dairies. A picture of the apparatus and carrying case is shown and list of items contained in the apparatus is given.

It is advantageous to use the portable resazurin outfit directly after milking as positive results on freshly-drawn milk indicate the presence of body cells or a mastitis condition. The more severe the mastitis the quicker is the dye reduced or changed from blue to pink. It is also advantageous to run the resazurin test on the mixed herd milk as it comes into the plant, for resazurin reduction in such milk would probably be due to bacteria.

For quick resazurin tests on the farm the sample can be milked directly into the test tubes after removing the foremilk. It is recommended that individual quarters be tested. The rennet test is recommended for use in conjunction with the resazurin test. The methods for performing these tests are outlined.

Very bad samples will reduce resazurin rapidly and fail to clot with rennet. If a sample clots slowly with rennet but does not reduce resazurin, it may indicate a past history of mastitis. A change of color of resazurin without slow clotting may indicate an incipient infection which should receive treatment at once.

The following advantages of testing cows for abnormal milk are listed:

- Economic advantages. Infected cows are inefficient converters of food to milk.
- Danger of low solids-not-fat. The writer claims that the resazurin-rennet test can be correlated closely with solids-not-fat content.
- Danger of infecting other cows. The resazurin test may pick out bad cases of infection which may be segregated or disposed of or milked last.
- Effect on bacterial count. Abnormal milk usually increases bacterial count of mixed milk from herd.
- Effect of abnormal milk on manufacture of milk products. Abnormal milk produces weak body and slow starter in cheese manufacture.

The test can be used to good advantage in checking animals that are being purchased for addition to the herd.

H.P.

184. **Interpreting Bacterial Counts to Producers.** O. A. GHIGGOILE, Chief, Bur. of Dairy Serv., Calif. State Dept. of Agr. *Milk Plant Monthly*, 32, No. 10: 26-27. 1943.

The responsibility of producing good milk rests with the producer, who

should be instructed in a non-technical language about bacteria, their mode of entry into milk, their rate of multiplication, and the cleanliness and care of dairy equipment. The manner in which the producer is approached will do much in bringing about cooperation in producing a better grade milk. Once the producer realizes and accepts his full responsibility in producing high quality milk with a low bacterial count, he will be proud of his accomplishment. G.M.T.

**185. How to Prevent and Remove Milk Deposits.** LEWIS SHERE, Pres., The Diversey Corp. *Milk Plant Monthly*, 32, No. 11: 32, 34, 39, 40. 1943.

Milk stone is a complex homogeneous mixture of organic and inorganic substances which adhere tenaciously to dairy equipment. It is unsightly, may cause off-flavors, interferes with heat transfer and is a source of high bacteria counts. The amount and composition of milk stone is influenced by: 1, the speed with which the milk flows through the equipment; 2, the final temperature to which the milk is heated; 3, the amount of milk handled; 4, type of dairy product handled; 5, type of equipment used; 6, hardness of the water; 7, nature of cleaners and sterilizers; and 8, time of rinsing.

Milk stone formation may be minimized by: 1. Rinsing the equipment with cold water; 2. Cleaning equipment thoroughly every day; 3. Rinsing the cleaning solution from the equipment; 4. Using chlorine sterilizers rather than heat; 5. Preventing corrosion of equipment.

Accumulated milk stone may be removed effectively and efficiently by using an acid-type product, following the manufacturer's directions. Precautions should be taken that the equipment is not damaged. G.M.T.

**186. Interpreting Bacterial Counts to Producers.** WALTER D. TIEDEMAN, Chief of Bur. of Milk Sanitation, N. Y. State Dept. of Health. *Milk Plant Monthly*, 32, No. 10: 25-26. 1943.

Bacterial examination of milk is done primarily to improve the quality of the raw milk supply. Interpretations of the bacteria count should be made in a language the producers understand by one who appreciates the limitations of the laboratory tests. Since there is no advantage in reporting figures, the reporting of the following classes of bacteria is suggested: class 1—200,000 or less; class 2—from 200,000 to 1,000,000; class 3—over 1,000,000.

Milk falling in class 1 is satisfactory; that in class 2 needs improvement; while that in class 3 calls for immediate and definite action.

A similar classification of cell counts is suggested as follows: cells += 500,000 or less per ml.; cells ++ = from 500,000 to 5,000,000; cells +++ = over 5,000,000.

A two-plus cell count might be interpreted as indicating the presence of mastitis in the herd, while a three-plus cell count is more definitely an indication of mastitis particularly when accompanied by long chain streptococci. The technician should help the dairyman by observing the type of bacteria and suggesting their significance. Repeating counts after unsatisfactory reports have been reported to see whether the report has been translated into action is good practice.

G.M.T.

## PHYSIOLOGY

187. **The Blood Volume of Normal Animals.** F. C. COURTICE. *Jour. Physiol.*, 102, No. 3: 290. Dec., 1943.

The blood volume of 30 goats has been estimated with the blue dye T-1824. The mean plasma volume was 53 cc. per kg. body weight and blood volume was 70 cc. per kg. The blood volume of goats as well as that of rabbits, dogs and horses is proportional to the body weight and not to surface area. The effect on blood volume of stage of lactation or productivity is not mentioned. The author does state that the blood volume of four highly trained greyhounds was much higher due to a higher cell volume. The blood volume depends upon the bulk of the animal tissue, especially muscle, and not upon the rate of metabolism.

D.E.

188. **Lipolysis and Fat Absorption.** A. C. FRAZER, *Physiol. Dept., St. Mary's Hosp. Med. School, London, and the Pharmacol. Dept., Univ. of London.* *Jour. Physiol.*, 102, No. 3: 329. Dec., 1943.

The ingestion of neutral fat normally leads to a characteristic appearance of the intestinal cells, to milky of the lacteals, to a marked systemic lipemia and to deposition of fat in the fat depots. The addition of potent lipase to the ingested neutral fat causes small instead of large granules to appear in the intestinal cells, the lacteals remain almost clear, the systemic blood shows but a slight lipemia, and the deposition of the fats is much decreased. The portal blood and liver, which show only slight changes after neutral fat ingestion, exhibit marked lipemia and deposition respectively if lipase is added to the neutral fat. The results following the ingestion of neutral fat and lipase are thus similar to those seen after the administration of fatty acid. It is possible to suppress almost completely the post-absorptive systemic lipemia by the addition of lipase to the standard fat-containing meal. Lipolysis should be regarded as a determining factor in the fate of absorbed fat and possibly as a means of providing essential raw materials for the synthesis of lecithin and the formation of soaps.

The complete inhibition of lipolysis by a long chain sulphate, sodium cetyl sulphate, in rats, does not prevent triglyceride absorption.

D.E.



189. **Differentiation in the Absorption of Olive Oil and Oleic Acid in the Rat.** A. C. FRAZER, Physiol. Dept., St. Mary's Hosp. Med. School, London, and the Pharmacol. Dept., Univ. of Birmingham. *Jour. Physiol.*, 102, No. 3: 306. Dec., 1943.

According to the author's view, lipolysis is only partial in the intestinal tract of the adult rat, and hydrolysis of the triglyceride molecule is not regarded as an essential preliminary to its absorption. Fatty acid passes by the portal vein to the liver, while neutral fat goes by the lymphatic route to the systemic blood and thence to the main fat depots to be stored for future use. Stained fatty acids fed over a period of 10 days result in no staining of these areas, but rather, appear in the liver. The degree of lipolysis is, thus, a determining factor in the immediate fate of absorbed fat.

D.E.



# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

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## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

190. **Simple Multiple-Loop Method Speeds Bacterial Counts.** ANDREW MOLDAVAN, Pure Milk Co., Montreal, Canada. *Food Indus.*, 15, No. 6: 56. June, 1943.

The author states that plate counts are time-consuming. ° In order to simplify bacteria estimates a modified Myers-Spence procedure is described.

It involves using a loop and melted cooled nutrient agar. Thus the operator is given a chance to do many determinations with a minimum of effort.

The apparatus is described. Loop and pipette accuracy is discussed, including a method for calibrating the loop with a 0.01-ml. pipette.

J.C.M.

191. **Research Finds Substitute for Bacteriological Agar.** W. E. BAIER AND T. O. MANCHESTER, Ontario. *Food Indus.*, 15, No. 7: 94. July, 1943.

A shortage of agar has caused investigators to search for a substitute for bacteriological agar. As a result, a pectinous material, sodium ammonium pectate, has been used successfully in some of the most commonly employed solid media.

Culture media for plates and slants, particularly counting plates considered here, demand a gel-producing constituent which meets very exacting specifications. Other gel materials have been suggested and some have been used in special applications, but agar has been and still remains the standard gel-forming agent for bacteriological culture media.

The article gives formulae for making the substitutes for agar for milk work. Details are given for using the substitute for agar. J.C.M.

### CHEESE

192. **New Way to Dehydrate Cheese.** GEORGE P. SANDERS, U.S.D.A., Washington, D. C. *Food Indus.*, 15, No. 10: 80. Oct., 1943.

American cheddar cheese usually contains more than 33% water, removal of which would reduce the weight by at least one-third, and would also make it possible to compress the cheese into a smaller volume. These savings are important under wartime shipping and storage conditions, and therefore the commercial dehydration of cheese has been undertaken.

Under the new method, which is applicable to any type of hard cheese, only properly cured cheese, selected with particular attention to flavor, and

thoroughly cleaned, should be used. This is blended as in the manufacture of process cheese. The subsurface portion of the rind, if clean and edible, can be used in the blend.

The author gives details of drying and compressing. Uses and quality as affecting the process are discussed. The author feels that the laboratory successes deem a commercial trial as being desirable. J.C.M.

## CHEMISTRY

193. **Chromatographic Determination of Carotene in Alfalfa.** L. W. CHARKEY AND H. S. WILGUS, JR., Colo. Agr. Expt. Sta., Fort Collins, Colo. Jour. Indus. and Engin. Chem., Analyt. Ed., 16, No. 3: 184. Mar., 1944.

Three important factors may cause errors in the determination of carotene in plant tissues. There may be oxidative losses of carotene, errors due to incomplete extraction from the tissue or incomplete separation of carotenes from other pigments. The chromatographic method described in this report, supported by experimental data, avoids these sources of error. The method includes an enzyme inactivation and sample storage procedure, making possible the collection and preparation of large numbers of samples on fixed dates. The chromatographic technique was modified by converting the adsorption column to an adsorption filter which avoided losses of adsorbed carotene. B.H.W.

194. **Yeast Microbiological Methods for Determination of Vitamins. Pantothenic Acid.** LAWRENCE ATKIN, WILLIAM L. WILLIAMS, ALFRED S. SCHULTZ, AND CHARLES N. FREY, Fleischmann Labs., Standard Brands, Inc., New York, N. Y. Jour. Indus. and Engin. Chem., Analyt. Ed., 16, No. 1: 67. Jan., 1944.

Pantothenate is determined by adding an extract of the unknown to 5 ml. of basal pantothenic acid-free medium in a test tube, heating, then cooling and inoculating the tubes with 1 ml. of the yeast, *Saccharomyces carlsbergensis*. The tubes are shaken at 30° C. for 16 to 18 hours and the yeast growth is estimated by turbidimetric measurements made directly on the tubes with the photoelectric colorimeter. The basal medium contains ammonium sulfate as a nitrogen source and in addition sufficient asparagine to prevent interference due to B-alanine. Extracts of substances to be assayed are prepared by aqueous extraction under pressure (15 pounds for 15 minutes) at pH 5.6 to 5.7, by enzyme digestion at the same pH, or by enzyme digestion followed by aqueous extraction (15 pounds for 15 minutes). The choice of extraction method depends upon the substance, since some have pantothenate in a bound form whereas others do not. The results

of assays of a number of substances including pasteurized milk, dry skim milk and whey compare favorably with results obtained by other methods.

B.H.W.

195. **Quantitative Determination of d-Galactose by Selective Fermentation with Special Reference to Plant Mucilages.** LOUIS E. WISE AND JOHN W. APPLING, Inst. of Paper Chemistry, Appleton, Wis. Jour. Indus. and Engin. Chem., Analyt. Ed., 18, No. 1: 28. Jan., 1944.

A method is described which permits the determination of small amounts of d-galactose in the presence of mannose, glucose, fructose, xylose, arabinose and glucuronic acid with an accuracy of 92% to 98%. It depends on differential fermentations with two yeasts, *Saccharomyces carlsbergensis* which ferments galactose and *S. bayanus* which does not ferment galactose. The yeasts have little effect on xylose, arabinose or glucuronic acid. The reducing values of galactose, mannose and d-glucurone were determined by the Munson-Walker method. The fermentation techniques were successfully applied to the hydrolysis products of pure lactose and certain plant mucilages.

B.H.W.

196. **Determination of Vitamin A and Carotenoids in Butterfat. Spectroscopic Characteristics of Butterfat Fractions and Problems Involved in Biological Interpretations.** F. P. ZSCHEILE, R. L. HENRY, J. W. WHITE, JR., H. A. NASH, C. L. SHREWSBURY, AND S. M. HAUGE, Purdue Univ. Agr. Expt. Sta., Lafayette, Indiana. Jour. Indus. and Engin. Chem., Analyt. Ed., 16, No. 3: 190. Mar., 1944.

Butterfat samples produced under different dietary conditions were studied by the direct spectroscopic method. Total carotenoids were estimated and ultraviolet measurements were made on the unsaponified fraction. Curves of the total carotenoids and of the carotene fraction from a series of butters were compared with that of  $\beta$  carotene. Corresponding curves of the unsaponifiable fraction in the ultraviolet region were compared with that of vitamin A. Effects of clarification, adsorption, acid extraction, and freezing upon the curves were studied, as well as various factors affecting the reliability of the experimental procedures. No clear-cut relationships could be established as a result of attempts to correlate spectroscopic and biological values. The feed of the cows had a great influence on the nature of the carotenoids present in the butterfat. There should be more extensive purification of the vitamin A fraction for the successful application of direct spectrophotometry to the determination of vitamin A in butterfats.

B.H.W.

197. **Fatty Acid Monoesters of l-Ascorbic and d-Isoascorbic Acids as Antioxidants for Fats and Oils.** R. W. RIEMENSCHNEIDER, J. TURER, P. A. WELLS, AND WALDO C. AULT, Eastern Regional Res. Lab., U. S. Dept. of Agr., Philadelphia, Pa. *Oil and Soap*, 21, No. 2: 47. Feb., 1944.

Fatty acid monoesters of l-ascorbic and d-isoascorbic acids were found to have antioxygenic activity in fats and oils. These substances were found to counteract the deleterious effect of traces of soap on the stability of fats. In combination with either  $\alpha$ -tocopherol or phospholipids or both, the ascorbyl monoesters exhibit marked synergistic antioxidant effect. Postulations as to the cause of synergistic phenomena are presented. J.L.H.

198. **The Antioxidant Properties of Nordihydroguaiaretic Acid.** W. O. LUNDBERG, H. O. HALVORSON, AND G. O. BURR, Univ. of Minn., Minneapolis. *Oil and Soap*, 21, No. 2: 33. Feb., 1944.

The antioxidant properties of nordihydroguaiaretic acid in lard are described. This phenolic-type inhibitor was obtained from a common desert plant (*Larrea divaricata*). Nordihydroguaiaretic acid exhibited synergistic action with ascorbic acid but not with a wheat germ concentrate containing 40% of mixed tocopherols. The effectiveness of the antioxidant in stabilizing lard is to some extent carried over into baked products (pie crusts).

J.L.H.

199. **Formation and Decomposition of Peroxides of Unsaturated Fat Esters.** R. F. PASCHKE AND D. H. WHEELER, U. S. Regional Soybean Industrial Products Lab., Urbana, Ill. *Oil and Soap*, 21, No. 2: 52. Feb., 1944.

The influence of temperature on the formation and decomposition of peroxides of unsaturated fat esters (distilled methyl esters of soybean fat acids) was investigated. The curves of decrease of iodine value and increase in peroxide value were found to parallel each other in the early stages of the reaction. As the temperature of oxidation increased above 35° C., the lower was the level at which the curves deviated and the lower the maximum peroxide value attained. At 15° and 35° C. the same maximum of peroxide values was observed, and at this point the value obtained was approximately 30% of what it would be if all the double bonds destroyed were converted to peroxides. The speed of decomposition of peroxides became progressively greater as the degree of oxidation increased. The rate of decomposition or disappearance of peroxide was found to agree best with that of a bimolecular reaction but definite exceptions were observed.

Investigation of reaction time and effect of oxygen on the determination of peroxides by the acetic acid-potassium iodide method, showed that a one-



hour reaction time in the absence of oxygen was necessary, especially on samples of high peroxide content. J.L.H.

**200. The Fluorescence of Chlorophyll in Fats in Relation to Rancidity.**

C. S. FRENCH AND W. O. LUNDBERG, Univ. of Minn., Minneapolis, Minn. *Oil and Soap*, 21, No. 1: 23. Jan., 1944.

The "chlorophyll value" as a measure of the keeping quality of oil was investigated. According to theory the color change was due to the "quenching" of the chlorophyll fluorescence by the transfer of excitational energy from chlorophyll molecules to acceptor molecules contained in the fat.

It appears that the disappearance of chlorophyll fluorescence in ultraviolet light is due to the absorption of the light by the cottonseed oil and to the intense white fluorescence of the oil itself rather than to a chemical reaction of some constituent of the oil with the excited chlorophyll. The lack of correlation between either the peroxide value or the conventional stability measurements and amount of chlorophyll fluorescence in the fats used makes the "chlorophyll value" test "appear to have doubtful value as a generally applicable test for fat rancidity or stability."

The absorption of near ultraviolet light by oxidized fats may be related to their content of fat peroxides. J.L.H.

**201. The Use of Refractive Index Measurements in Fatty Acid Ester Analysis.** KARL F. MATTIL AND HERBERT E. LONGENECKER, Univ. of Pittsburgh, Pittsburgh, Pa. *Oil and Soap*, 21, No. 1: 16. Jan., 1944.

The determination of the composition of fats and oils is usually accomplished by the fractional distillation of their mixed methyl esters and the analysis of the separated fractions. When packed columns are used, fractions containing not more than two adjacent homologous components can be obtained. The fractions can then be analyzed for composition by the use of simultaneous equations based on the saponification equivalent, the iodine value and the thioeyanogen value. Two inherent difficulties of the method are, (a) the accuracy with which the analytical constants must be determined and, (b) the small size of certain fractions (0.1 to 0.2 grams).

The linear relationship that exists between the refractive index and the composition of a mixture of adjacent homologous methyl esters makes it possible to use the index as a tool in the calculation of the composition of unknown mixtures. For precise work it is necessary to use a refractometer where the fifth decimal can be estimated and where the temperature can be controlled or known to a few hundredths of a degree. One important advantage of the method is that the analysis can be completed soon after the fraction is taken from the column. J.L.H.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

202. **Compressing Spray-Dried Milk to Save Shipping Space.** B. H. WEBB AND C. F. HUFNAGEL, U. S. Dept. of Agr., Washington, D. C. Food Indus., 15, No. 9: 72. Sept., 1943.

Studies show that as much as 42% of the space occupied by dried milk can be saved by compression, 24% by jolting. Milks subject to different manufacturing conditions, however, may vary widely in their compression characteristics.

Important savings in the amount of shipping space taken up by spray-dried milk can be attained by compression and by jolting or bumping in wartime packaging operations. The savings in space effected by compression may be expected to range from approximately 21% for compression in a small package at a pressure of 500 lb. per square inch to 42% for die compression at 3,200 lb. per square inch. Jolting may be expected to save 11% of the space in a bulk package and 24% in a small package.

The details of both procedures are given, and the advantages listed.

J.C.M.

203. **Removal of Oxidizing Factors Makes Dry Whole Milk Keep.** C. D. DAHLE AND D. V. JOSEPHSON, Penn. State College, State College, Pa. Food Indus., 15, No. 11: 76. Nov., 1943.

Whole milk powder can be treated in processing so as to remove pro-oxidant constituents which make it subject to oxidized or tallowy flavor. The product then has excellent keeping qualities and should satisfactorily meet present requirements.

That whole milk powder develops a stale oxidized flavor after some weeks or months of storage is a fact which has hindered this product from gaining wide acceptance. But the present war has focused attention again on dried whole milk because huge amounts are purchased by the military and lend-lease authorities. No doubt it will prove to be in great demand, even after actual fighting stops.

The authors feel that oxidation of dried whole milk can be improved when better procedures are available to do on a large scale these things which were achieved in a laboratory.

J.C.M.

## ICE CREAM

204. **Conserving Milk Solids.** J. H. ERB, The Borden Co., Columbus, Ohio. Ice Cream Trade Jour., 40, No. 3: 20. March, 1944.

It is pointed out that considerable improvement in the quality of "war-time ice cream" is possible, without the substitution of cereal products for lacking milk solids, if the manufacturer makes use of present knowledge and

gives close attention to each step of processing. The following suggestions are made:

Superheated condensed milk should be used rather than unsuperheated, and frozen condensed milk should be avoided. Efficient homogenization is very important. Low solids mixes should be aged 24 hours. The proper quantity of a good stabilizer should be used and it should be handled to get the maximum benefit from it. Mix should be quickly frozen to as stiff a consistency as possible and hardening should be rapid. F.J.D.

**205. Velva Fruit—A New Frozen Fruit Dessert.** H. J. LOEFFLER, Western Regional Res. Lab., U.S.D.A., Albany, Calif. *Ice Cream Trade Jour.*, 40, No. 3: 16. March, 1944.

The author describes a new frozen dessert made from fruit purees. The name "Velva Fruit" is suggested to distinguish the new product from ices and sherbets. Suitable purees can be prepared from a large number of different fruits and some blends. The whole, fleshy, fruit is used in preparing the purees which may then be frozen for distribution to the manufacturer, or they may be used without freezing if kept cold and utilized in a relatively short time.

To the puree is added sugar, gelatin and perhaps citric acid if the fruit is not sufficiently tart. The amount of sugar is varied somewhat with the sweetness of the fruit and about 0.6% of gelatin is usually required. The mixture is frozen in ice cream equipment to approximately 100% overrun. Several formulas are given.

"Velva fruit" differs from ices and sherbets in that the fruit solids and juice account for 60% of the finished product as compared with about 20% where the fruit is used primarily as a flavor. The overrun is also considerably higher but the extra fruit solids prevent excessive fluffiness. "Velva fruit" melts somewhat slower than ice cream but has similar body or dipping qualities at similar temperatures. F.J.D.

**206. An Excess Solids Method for Calculating Ice Cream Mixes.** ALAN LEIGHTON, Bur. of Dairy Indus., Washington, D. C. *Ice Cream Rev.*, 27, No. 3: 24. Oct., 1943.

The author has worked out a variation of the serum point method for calculating ice cream mixes. This method uses the water content of the mix and dairy products, instead of their serum as the reference medium. The method is explained in detail with examples. J.H.E.

**207. Use of Whey Solids in Ice Cream and Sherbets.** ALAN LEIGHTON, Bur. of Dairy Indus., Washington, D. C. *Ice Cream Rev.*, 27, No. 6: 18. Jan., 1944.

Whey solids from Cheddar cheese whey was used in experimental ice cream mixes to replace varying percentages of serum solids. In the case of

10% butterfat and 8% serum solids mixes, 1.6% of whey solids could be substituted with no detriment to quality.

When whey solids were added to mixes to raise the total solids content there was marked improvement in body and texture. No undesirable flavors were noted.

The experiments indicated that whey solids could be used advantageously in wartime ice cream.

Sherbets in which whey solids were used in place of normal milk-solids-not-fat were not noticeably different from the controls.

When using liquid whey it should be pasteurized to inactivate the rennet and thus avoid coagulation. J.H.E.

208. **Merchandising Ice Cream in War Time.** GEO. W. HENNERICH, Ice Cream Merchandising Inst., Washington, D. C. *Ice Cream Rev.*, 27, No. 3: 70. Oct., 1943.

The idea is expressed that ice cream merchandising is needed now more than ever. A program of merchandising must be carried out that will keep consumer interest in ice cream until consumer demand can again be supplied.

J.H.E.

209. **Quince Seed Extract as an Ice Cream Stabilizer.** GIDEON HADARY AND H. H. SOMMER, Univ. of Wis. *Ice Cream Rev.*, 26, No. 11: 22. June, 1943.

The properties of quince seed water extract as a stabilizer for ice cream have been studied and compared with those of gelatin. Quince stabilizer was found to dissolve readily in ice cream mix at a very wide range of temperatures. The action of quince upon mix viscosity was immediate, in contrast to gelatin which causes an increase with time. Mixes stabilized with 0.032% quince developed much less viscosity than similar mixes stabilized with gelatin.

Quince stabilized ice cream did not melt as uniformly as did those stabilized with gelatin.

At the present time quince seed must be imported and its high cost makes prohibitive its use as an ice cream stabilizer. J.H.E.

## MILK

210. **Safeguarding the Public Milk Supply in War Time.** C. J. BABCOCK, Major, Sanitary Corps, Army Service Forces. *Milk Dealer*, 33, No. 6: 31-32, 72-74. March, 1944.

The problems involved are the same as in peace time but complicated in that loss of enforcement personnel, population shifts, establishment of army camps, lack of processing machinery and availability of milk have been

retarding factors in supplying adequate and safe milk for particular states, cities and towns. Importation of milk from outside sources frequently involved lowered quality standards which the Army, through the Surgeon General and the Quartermaster General charged with safety of Army milk supplies, could not condone. A system of joint control and cooperation between the Army and local control authorities has largely solved the problems involved and assured both Army and civilian personnel of a safe milk supply—made possible by importation of milk from areas of greater production and by extending local milk sheds.

Lack of sufficient control officials has made routine farm inspections difficult if not impossible. Greater emphasis must therefore be placed upon platform inspection and rigid plant control of all processing methods such as daily pasteurization and *B. coli* tests to insure safety. "The dairy industry has not been forced (in the past) by regulations to improve the quality of milk, other than that used for fluid purposes." Herein lies the big problem of obtaining greater satisfactory supplies of milk for fluid use due to general lack of quality in milk used for processing other dairy products—a postwar as well as an immediate problem if the dairy industry is to survive. The need of better butter quality is cited as an example if markets temporarily lost to butter substitutes are to be regained after the war. Local politics, lack of knowledge in control officials and slipshod inspection methods attest to lack of adequate control in many sections—sufficient in some cases to justify complete Army control to the exclusion of local control. The author stresses the need of qualified and specially trained milk control officials in every community and not merely the drafting of milk ordinances whereby a Grade A cap may be placed on a bottle of milk. Tribute is paid to the milk industry for the job it has done in producing a safe milk supply under many handicaps and in creating milk drinking habits in our soldiers. "This milk drinking habit will return with the soldier to the civilian and the per capita consumption of milk will be higher than ever before in history."

C.S.T.

211. **Preventing Defects in Bottled Milk and Cream.** E. L. FOUTS, Dairy Technol., Agr. Expt. Sta., Gainesville, Fla. Milk Dealer, 33, No. 5: 29-30, 62-64. Feb., 1944.

The preventing of defects in bottled milk and cream has always been of vital importance in dairy plants. Under wartime conditions, extra problems are added to dairy plant management and merit closest attention. Listed as added problems for maintenance of quality are: (1) New milk supplies from producers not before qualified to sell milk for fluid consumption. Lack of proper equipment and information on production and handling of such milk needs careful study and cooperation on part of dealers and regulatory officials. (2) The problem of inexperienced employees in the

plant complicate the milk plant's problem. Special instruction and short courses for new workers are suggested as a remedy. (3) Lack of equipment and replacement machinery affect possible defects of milk and cream. Retinning and careful check and repair of all machinery is essential. (4) Less frequent delivery, while advantageous from cost of delivery standpoint, necessitates extra care in providing milk and cream free from flavor and physical defects. Flavor defects to guard against under wartime conditions are (1) oxidized flavor and rancidity, (2) bacteriological contaminations through lack of proper care from cow to container, (3) coliform organisms and (4) poor keeping quality. Eternal vigilance in all processing and handling steps will do much to control and reduce such defects in bottled milk. The defects to avoid in bottled cream of 19% are (1) cream plug, (2) oiling off, (3) feathering and (4) lack of viscosity. To avoid, use fresh cream, handle carefully, heat and cool rapidly, age 24 hours at 35 to 40°, and bottle and sell promptly. C.S.T.

212. **Postwar Milk Distribution Possibilities.** E. J. MATHER, Exec. Vice-Pres., National Dairy Products Corp. Milk Dealer, 33, No. 35: 33-34. Feb., 1944.

"More people are drinking more milk" than ever before in the United States. This increase has been brought about by the boost given milk consumption by the Army, defense plants, lend-lease and nutrition specialists. Tomorrow's problem is to preserve our present per capita intake of milk. The author stresses need of emphasizing the distinctive flavor of milk as inherent in milk from healthy cows fed correctly and the milk kept free from contamination from the cow to the table. Proper equipment for so producing and handling milk on the farm should therefore be stressed in the dairy equipment supply field. The responsibility of retaining wartime consumptive demands in postwar period is up to the farmer, the supply man and the processor of milk and milk products with economies of present production, procurement, processing and distribution continued and further reductions in manufacturing costs, labor and in equipment to the greatest extent. Then will volume be maintained at lower costs but still at a profit. C.S.T.

213. **Influence of Cooling Methods on Bacteria in Milk.** T. G. ANDERSON AND JOHN E. NICHOLAS, Penn. State Col. School of Agr. Bul. 454. Sept., 1943.

The milk used in this study was that produced by the college herd. Initial temperature of the milk at the time it was inserted in the cooler varied from 80.5° to 96° F. with an average of 90° F. Temperature measurements of the milk and cooling water were made with thermocouples. When cooling milk with well or spring water it is necessary that the water

temperature not rise higher than 50° F. to produce satisfactory results. Running water should have a temperature of 48° or less. When using ice at least five pounds per gallon of milk are necessary. Ninety per cent of the bacteria in milk are concentrated in the cream layer, two hours after milking. The bacteria remain evenly distributed throughout in the case of homogenized milk.

P.H.T.

214. **Milk and Cream Nomograph.** D. S. DAVIS, Wyandotte Chemicals Corp., Wyandotte, Mich. *Food Indus.*, 15, No. 12: 75. Dec., 1943.

This is a graphic interpolation of data on the dependence of solids-not-fat and specific gravity on butterfat percentages in milk and cream. J.C.M.

215. **Morale on the Milk Route.** VIRGIL M. BENEDICT, David's Dairy, Sturgis, Mich. *Milk Dealer*, 33, No. 5: 26, 66-68. Feb., 1944.

Morale is defined as "that something which urges one to give all that he or she has to achieve an objective." The author lists five points, honesty, reliability, business acumen, fairness and pride, as essential on the part of the employers of milk route salesmen if success in selling milk is to be attained. Without these qualities coupled with high morals and character the owner of a milk plant will be unable to build and sustain a high morale in the employee engaged in distributing and selling the company's products—milk and good will.

C.S.T.

## MISCELLANEOUS

216. **Maintaining Equipment at Peak Efficiency.** B. E. SAVEY, Borden's Dairy and Ice Cream Co., Columbus, Ohio. *Milk Dealer*, 33, No. 6: 104-112. March, 1944.

Proper maintenance reduces repair and requires careful planning, cooperation and a "follow-up" together with rigid adherence to operating manual of each piece of equipment as furnished by the manufacturer and a control system and record of all lubrication, repairs needed or effected, breakdowns or changes made. The factors to consider and stress in maintaining peak efficiency in a dairy plant are (1) proper and careful cleaning of all equipment by all employees; (2) care and repair of pasteurizers; (3) check gauge, packing, oil, valves and drive on homogenizers; (4) check float control, temperatures and scale accumulation on all coolers; (5) pumps should be regularly inspected for packing, gaskets, couplings, covers, pipe connections and motors; (6) clean, oil, check load, keep covered, rewind and overhaul all motors regularly; (7) check wear, grease, gears and replacement parts on all gear boxes; and (8) thoroughly check and inspect all ammonia compressors and condensers in order to avoid a slow-up or breakdown in refrigeration system. Eternal vigilance with all equipment is the keynote

of continued operation under wartime conditions in every dairy products plant.  
C.S.T.

217. **Aerodynamic Fly Control.** GERALD E. ZICH, N. J. Dept. of Agr., Trenton, N. J. *Milk Dealer*, 33, No. 6: 29. March, 1944.

A method whereby a current of air, generated by a quarter-horsepower motor blower placed over each doorway, chute or opening is directed downward to curtain the entire opening is described. The air is trained by the angle of a metal flange and then deflected outward at the bottom, blowing the fly or insect back into space instead of into the bottling plant. Diagrams illustrating installation and operation accompany article on this fly control measure said to be effective in eliminating flies in dairies and in milk plants.  
C.S.T.

218. **Postwar Planning in the Dairy Industry.** MERRILL O. MAUGHAN. *Milk Dealer*, 33, No. 6: 76-82. March, 1944.

Changing conditions brought about by the war will force the dairy industry to face many new problems. The things to expect as an aftermath of war are listed as follows: 1. Great excesses of goods in all fields with resultant increased competition between industries and between individual concerns. 2. Decreased earnings and reduced working hours, cushioned however by accumulative shortages in many consumer goods. 3. Continued government control of many industries to better effectuate diversion from war to peace status. 4. High consumption for dairy products due to increased emphasis upon nutritional value of all dairy products and upon increased demand of soldiers returning to civilian life "sold" on the value of dairy products. 5. Continued high taxes will prevail. 6. Strong labor unions will continue as a factor in all industries.

"Postwar planning should become postwar preparedness." Suggestions for the dairy industry to adopt now are as follows: 1. Plan for extensive promotion of all dairy products—a National Dairy Council unit in every state and principal market. Plan for both domestic and foreign markets. 2. Plan for extensive research to develop new uses, marketing methods and advertising. 3. Plan for more group action by forming and supporting strong trade associations. 4. Retain good features of war-imposed restrictions and eliminate bad of pre-war practices. 5. Stress quality for all dairy products. 6. Provide for utilization of surplus fluid milk. 7. Return to the realm of economic reality. 8. Plan for greater efficiencies in procurement, labor, processing and sales. 9. Improve our relations with the public. 10. Think in terms of the welfare of the entire industry. "Let's keep faith with each other and in our democratic form of Government."  
C.S.T.



219. **Some Suggestions for Keeping Those Trucks Rolling.** J. N. BAUMAN, White Motor Co. *Ice Cream Rev.*, 27, No. 6: 21. Jan., 1944.

Three fundamental things must be carried out if trucks are to be kept in efficient operation. These are (1) adequate and correct maintenance, (2) availability of parts when needed, and (3) proper care of truck equipment by the driver. J.H.E.

220. **Manual of Dairy Detergents and Cleaning Practices.** M. E. PARKER, Beatrice Creamery Co., Chicago, Ill. *Food Indus.*, 15, No. 7: 78. July, 1943.

The attributes and shortcomings of the various types of dairy washing compounds are cited and directions for cleaning cream cans, separators and farm utensils are given.

Effective cleaning of cream transport cans, separator parts and farm utensils is not attained by the mere use of washing compounds and chemical sterilizers. The purpose of any dairy cleaner is to prepare dirt, milk solids and grease for its subsequent detachment by brushing and its final elimination by rinsing.

As with many things about which dairymen have little exact knowledge, many dairy washing compounds sometimes are invested with magical powers. Their function is to remove dirt and grease. This is all any good cleaner can do. Differences in price may be based on the different qualities and combinations of the chemicals used, and other materials added to combat hardness in water or to give the cleaner some special character.

Selecting the right cleaner would be a simple matter if soft water were available everywhere. The degree of hardness in water varies in different parts of the country, even from town to town in some sections.

The various kinds of washing compounds available for dairy cleaning purposes may be classified generally as follows:

1. The alkalies and alkaline salts, such as caustic soda, sodium metasilicate, trisodium phosphate, sodium carbonate and bicarbonate of soda, or various mixtures of such chemicals.

2. The acid materials used for waterstone and milkstone removal, such as inhibited muriatic acid (hydrochloric acid), phosphoric acid, tartaric acid, as well as the new acid cleaners for general cleaning developed within recent years.

3. Natural materials such as wetting agents which rely on neither acidic or alkaline properties in their use.

4. The water-conditioning chemicals commonly referred to as the polyphosphates, which in general have no marked detergent characteristics but do have special properties in the compounding or application of effective cleaner mixtures, nevertheless.

5. Miscellaneous materials such as abrasives, metal cloth, and so forth, which are used as mechanical aids with or without cleaning compounds.

J.C.M.

221. **What's Ahead for Private Motor Trucks?** JOSEPH B. EASTMAN, Dir., Office of Defense Transportation. *Milk Dealer*, 33, No. 5: 31, 88-89. Feb., 1944.

Three factors stand out in private motor truck transportation. (1) Dependency of domestic economy and war effort on motor truck transportation. (2) The extent of loss of rubber for truck tires. (3) Competition of private trucks for wartime equipment, repairs and tires. In 1941, 700,000 motor truck units were sold as compared with 100,000 units in 1942 and 1943 but illustrates the complicated problems involved in continuing to operate, maintain and conserve motor truck transportation in the face of increased demands. Office of Defense Transportation's certificate of war necessity records shows a 20% mileage saving in truck operation despite increased demands. Private trucks have accomplished great savings by elimination of extra delivery, by consolidation of routes, greater loads, etc., and have effected in individual cases as much as 40% savings. These savings have not been made by all private trucks and a plan is made for 100% cooperation if private motor trucks are to continue to carry their fair share of wartime hauling.

C.S.T.

222. **Mechanical Treatment Destroys Insects in Foods.** E. S. STATELER, Food Indus., New York City. *Food Indus.*, 15, No. 7: 82. July, 1943.

Under peacetime conditions, the estimated annual loss of \$600,000,000 is an exorbitant toll to pay in food and grain supplies because of insect infestation. Under present conditions, that loss, which may become even greater because of handling, storage and shipping difficulties, is more serious than the mere monetary value involved.

The article contains procedures and precautions which are of value to anyone interested in processing food materials.

J.C.M.

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New York Association of Dairy and Milk Inspectors	United States Department of Agriculture

## ABSTRACTS OF LITERATURE

### BOOK REVIEW

223. **Biochemistry of the Fatty Acids—And Their Compounds, the Lipids.** W. R. BLOOR, Prof. of Biochemistry and Pharmacology, Univ. of Rochester, Rochester, N. Y. Reinhold Publishing Co., New York, 1944.

The author has made a distinct contribution to the biochemical field in this monograph because he has brought together in one volume the present information concerning the fatty acids and their related compounds. The organization of the material is well carried out. Chapter I. Chemistry: Descriptive and Analytical, covers Classification of Lipids, Physico-Chemical Conceptions of the Lipids, and Macro and Micro Methods under Methods of Examination of Tissue Lipids. Chapter II. Digestion and Absorption, comprises Nutritional Availability of Fats, Lipid-splitting Enzymes, Fat Digestion, Absorption of Fats, and Digestion and Absorption of Other Lipids. Chapter III. Lipids of the Blood, contains such items as Normal Basal Levels of Blood Lipids, Changes in the Post-absorptive Level Produced by Food, Variations in Blood Lipids in Normal Lipids, Effect of Abnormal Conditions on the Blood Lipids, Infections, Mental Disease, Organic Diseases, and Effect of the Lipids of the Blood on its Properties. Chapter IV. The Lipids in Tissue, lists Data on Tissue Lipids, Tissue Lipids in Abnormal Conditions, Nature and Function of Tissue Lipids, Lipids of Plants, and Lipid of Microorganisms. Chapter V. Lipid Metabolism, is devoted to metabolic and catabolic features, such as Physiological Synthesis of the Lipids, Intermediary Metabolism and the Role of the Liver, Catabolism of the Fats, Fat Metabolism in the Developing Embryo, and the Vitamins in Lipid Metabolism. The last chapter, Chapter VI. The Lipids of Secretions and Excretions, takes up under Food for the Embryo and Young Organism, The Placenta, Milk and Eggs. In addition Lymph and Chyle, Cerebrospinal Fluid and Bile are considered, and finally Excretions. Each chapter is followed by an extensive bibliography varying in length from about three to seven and one-half pages. L.M.D.

### BACTERIOLOGY

224. **The Burri Slant Technique in the Food Industries.** C. K. JOHNS, Dept. of Agr., Ottawa. Food in Canada, 4, No. 3: 17. 1944.

A test first described by Burri in 1928 can be often used to examine dairy products and other foods, in place of more expensive and complicated methods. The medium recommended is tryptone glucose skim milk agar

with brom-cresol purple added. The use of an additional 0.5% agar is suggested and an oval rather than a round tube may be preferable. The size of the inoculating loop may be varied to accommodate the number of organisms. O.R.I.

225. **Few Bacteria in Canadian Eggs.** C. K. JOHNS, Dept. of Agr., Ottawa. *Food in Canada*, 4, No. 4: 16. 1944.

This author had previously reported on the numbers of bacteria found in storage eggs. The present study was done on fresh eggs and indicates that low counts are commonly found. Of 219 eggs examined, 77% had counts of less than 100 organisms per gram. O.R.I.

## CHEMISTRY

226. **The Antimony Trichloride Method for the Determination of Vitamin A.** G. H. BENHAM, McGill Univ., Montreal. *Canad. Jour. Res., B*, 22, No. 2: 21. 1944.

A critical description of the antimony trichloride method for the determination of vitamin A is presented. Low values for vitamin A result from:

- (1) Incomplete extraction from the alcoholic soap solution by using petroleum ether instead of ethyl ether.
- (2) Incomplete separation of the layers during extraction and washing.
- (3) Incomplete filtration through anhydrous sodium sulfate.

If strict attention is paid to details of procedure, the method gives consistent results. Uncertainty in regard to the exact factor for converting the *E* values to international units makes it impossible at this time to state accurately the absolute values. It is pointed out that this in no way detracts from the usefulness of the chemical test. O.R.I.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

227. **Concentrated Dairy Products.** J. L. PERLMAN AND A. H. ROBERTSON, Dept. of Agr. and Mkts., Albany, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 43, 1943.

The use of neutralizers to conceal inferior quality products in concentrated dairy products is an old practice that is deplorable. Neutralizers are used alone and in combination with sirups, stabilizers, flavors, etc. Special consideration is given to frozen desserts. Methods of detecting neutralizers are not simple and one of the best known is the Hillig method based upon the alkalinity of the ash. Data are presented on frozen desserts to show that this method detects as little as 0.05% sodium bicarbonate in milk and 0.1% in heavy cream. A.C.D.

## DISEASE

228. **Bovine Brucellosis.** ANONYMOUS. Univ. of Ill., Col. of Agr. Cir. 573. 4 pages. March, 1944.

This leaflet lists nineteen "don't's" to be observed in developing and maintaining a milking herd free from Brucellosis (Bang's disease).

J.G.A.

229. **Vitamin-D Deficiency in Dairy Cows.** G. C. WALLIS. S. Dak. Agr. Expt. Sta. Bul. 372. 16 pages. March, 1944.

Symptoms, causes, and treatment are outlined in some detail.

How much vitamin-D deficiency there is in dairy cattle is not known. Cows on pasture during the summer ordinarily build up vitamin-D reserves due to exposure to the direct rays of the sun. When they are then fed during the winter on sun-cured roughages high in vitamin D, they probably receive more than 12,000 to 15,000 International Units of vitamin D daily. These amounts are about enough, according to studies by the author. However, even then milk production, calving records, and general health might be improved during the winter by some additional vitamin D.

On the other hand, there are undoubtedly many times when roughages low in vitamin D are fed. Then when spring comes, cases of mild vitamin-D deficiency and lowered general health and producing ability may occur.

Further information is needed to determine the vitamin-D content of various roughages and the degree of vitamin-D deficiency that may exist in dairy cows in winter. Until this information is available, a farmer will do well to be sure that his cattle get generous amounts of sun-cured hay and that they are exposed to sunshine often, particularly during the summer.

J.G.A.

230. **A Bromthymol Blue Field Test for Bovine Mastitis.** FRANCIS J. HALLINAN, N. Y. State Dept. Health, Albany. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 121, 1943.

The usual procedure for detecting mastitis by the bromthymol blue method is subject to certain errors that can be corrected. The author presents a simple procedure for field test using reproducible permanent standards prepared from Munsell color papers.

A.C.D.

231. **Augmenting War-time Milk Production by Converting Cows Condemned for Mastitis to Useful Three Teaters.** F. W. GRAVES, N. Y. State Dept. of Health, Albany. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 11, 1943.

Under war-time conditions much milk is lost unnecessarily by condemnation of three-teated cows. If such cows can be saved, there will be a better

opportunity to cull unprofitable cows. The Federal Meat Inspection Service does not condemn the entire carcass because one area is infected; the same policy can hold true for milk inspection.

If a quarter is infected, it should be dried up. If it dries up well it may never milk again. There is also the chance that it may give good milk when the cow freshens. If completely dry the three-teater is a safe cow. If the infected quarter discharges milk it should be treated by a veterinarian to dry it permanently. This is done by passing the fumes of 4 ounces of ether directly into the infected quarter and after one month removing such material as can be withdrawn by milking. The quarter will be permanently dry. A.C.D.

## FEEDS AND FEEDING

232. **Single Grains and Grain Mixtures as Supplements to Alfalfa Hay and Silage for Milk Production.** J. R. DAWSON, A. L. WATT, C. W. MCINTYRE, R. E. LEIGHTON, AND R. R. GRAVES. U. S. Dept. Agr. Cir. 696. 11 pages. Feb., 1944.

At each of three field experiment stations, two groups of four cows each were fed unlimited quantities of alfalfa hay and silage throughout their lactation periods of 365 days. In addition to this basic ration of roughage, one group of cows was fed a single grain—ground barley, ground corn, or ground kafir—at the rate of approximately 1 pound to each 6 pounds of milk they produced. The other group was fed a grain mixture, at approximately the same rate, which consisted of four to six different grains, grain by-products, and high-protein concentrates.

The cows receiving the single-grain ration had somewhat better breeding records during the experiment, gained more weight, and produced 95% as much butterfat as the cows on the mixed-grain rations. However, the results were not consistent and the difference in production therefore is probably not significant.

This experiment does not indicate the relative value of a single-grain ration and a mixed-grain ration when the roughages are so restricted that a large proportion of the nutrients must be furnished by the grain part of the ration. It does show that where cows have an opportunity to consume as much good hay and silage as they like, it makes little difference whether the additional nutrients they require are obtained from a single grain or a mixture of several grains and grain by-products.

At current market prices the cost of the feed required to produce a pound of butterfat averaged slightly less for the single-grain rations than for the mixed-grain rations. J.G.A.

233. **Carotene Requirements for the Maintenance of a Normal Spinal Fluid Pressure in Dairy Calves.** L. A. MOORE, M. H. BERRY, AND



J. F. SYKES, Dairy Dept., Univ. Maryland, College Park, and Dept. of Physiol., Mich. State Col., East Lansing. Jour. Nutr., 26, No. 6: 649-658. Dec., 1943.

Two groups of calves (Holstein and Ayrshire) were used to study the amount of carotene required to maintain a normal spinal fluid pressure. One group was located at East Lansing, Michigan, and the other at College Park, Maryland. Alfalfa meal was used as a source of the carotene supplement. Ophthalmoscopic observations were made periodically. Blood plasma vitamin A and ascorbic acid values were also determined.

An intake of 66 micrograms per kilogram of body weight during the winter months is about the minimum requirement for carotene for Holstein and Ayrshire calves when spinal fluid pressure is used as a criterion. Because of individual characteristics, plasma vitamin A or carotene values will not distinguish between variations of carotene intakes of 62 to 75 micrograms per kilogram of body weight. C.F.H.

**234. The Use of Urea in Making Silage from Sweet Sorghum.** A. E. CULLISON, Miss. Agr. Expt. Sta., State College, Miss. Jour. Anim. Sci., 3, No. 1: 59-62. Feb., 1944.

Silage made from freshly cut sweet sorghum treated with about 10 pounds of urea per ton was compared with a lot untreated. The addition of urea speeded up the rate of fermentation, saved carotene and produced a silage with greater titratable acidity.

In a feeding trial with beef cows the urea-treated silage proved more palatable than the untreated. The cows that received the urea-treated silage maintained their weight while the untreated lot lost 47 pounds over a 78-day period. The urea-treated lot of cows were much better in appearance. C.F.H.

**235. The Effect of Fat upon the Digestion of Nutrients by Dairy Cows.** H. L. LUCAS AND J. K. LOOSLI, Cornell Univ., Ithaca, New York. Jour. Anim. Sci., 3, No. 1: 3-11. Feb., 1944.

Two series of digestion trials using Holstein cows were conducted with rations of varying fat contents. In series one, there was no difference in the apparent digestibility of rations containing 1.6 and 2.6% ether extract.

In series two, using soybean products as the sole concentrates, rations containing 1.0 and 7.0% of ether extract were studied. The crude fiber and nitrogen-free extract of rations containing soybeans and solvent extracted soybean oil meal plus corn or soybean oil were less digestible than where the rations contained solvent-extracted soybean oil meal. The ether extract of rations containing soybean oil meal plus oil or fatty acids was more digestible than the ether extract of ration containing soybeans.

The ration containing soybeans caused a marked increase in fat percentage with practically no change in milk production. On the other hand, the rations containing either oil or fatty acids caused a pronounced decrease in milk flow, with little change in fat percentage. C.F.H.

## FOOD VALUE OF DAIRY PRODUCTS

236. Further Studies on the Comparative Value of Butterfat, Vegetable Oils, and Oleomargarines. R. K. BOUTWELL, R. P. GEYER, C. A. ELVEHJEM, AND E. B. HART, Dept. of Biochem., Univ. of Wis., Madison. *Jour. Nutr.*, 26, No. 6: 601-609. Dec., 1943.

When lactose was the sole carbohydrate in the diet, rats showed superior growth with butterfat or lard as compared to corn oil, coconut oil, cottonseed oil, soybean oil, peanut oil, olive oil and hydrogenated cottonseed oil. When a mixture of carbohydrates composed of sucrose, starch, dextrose, dextrin and lactose was in the diet, the average growth of the animals fed vegetable oils was equal to that of the animals fed butterfat or lard.

Properly fortified oleomargarine fats gave growth equal to butterfat over a period of 6 weeks with the above mixture of carbohydrates. Butterfat was superior to fortified oleomargarine when lactose was the sole carbohydrate in the diet. C.F.H.

237. Studies of the Comparative Nutritive Value of Fats. I. Growth Rate and Efficiency of Conversion of Various Diets to Tissue. H. J. DEUEL, JR., E. MOVITT, L. F. HALLMAN, AND F. MATTSO, Biochem. Dept., Univ. Southern Calif., Los Angeles. *Jour. Nutr.*, 27, No. 1: 107-121. Jan., 1944.

In three series of tests using male and female rats for periods of 6 to 12 weeks, the following fats were compared: a butterfat or whole butter, corn oil, cottonseed oil, or margarine fat or a whole margarine, olive oil, peanut oil or soybean oil. The basal diets used consisted of mineralized skimmed milk powder, with vitamins A, D and E. In one series whole butter and margarine were flavored by the addition to the fats of 4 parts per million of diacetyl.

In general the rats preferred a butter diet to one containing the vegetable fats. There was no difference, however, in the growth of weanling rats over a 12-week period when they were fed mineralized skimmilk powder, vitamin supplements and butter as compared with corn, cottonseed, olive, peanut or soybean oils or margarine. Also, the efficiencies of conversion of these various fats to body tissue were identical. The authors concluded that these results refute the idea that butterfat possesses certain saturated fatty acids not present in other fats, which are essential for growth. C.F.H.

## ICE CREAM

238. **Frosted Foods and the Ice Cream Industry.** R. M. LAMBETH, Grand Rapids Cabinet Co. *Ice Cream Field*, 43, No. 4: 34, 54, 56. 1944.

The author states that the first year following the war 150,000 ice cream cabinets will be built. He further predicts that there will be a buyer for all the cabinets produced for five years following the war. This prediction is made on the basis that a home freezer or storage cabinet is practical, economical and a convenient way to preserve foods.

It is stated that the past year has seen a large increase in demand for ice cream cabinets for the storage of frozen foods—many have been bought for the purpose of putting up “victory” garden products.

It is predicted that eventually “frozen foods will dominate the food field,” and that the greater availability of cold storage cabinets will influence the future methods of distribution of ice cream.

It is claimed that there are 7,500 locker plants in the United States with 3,250,000 individual lockers which serve 3,000,000 families.

When restrictions on deliveries and equipment are removed the author expects there will be a marked increase in frozen food activity, including home delivery of such foods. He claims the dairy industry and especially the ice cream industry, has a distinct advantage in this field. W.C.C.

239. **The Melt Test.** B. I. MASUROVSKY, Res. Ed. *Ice Cream Trade Jour.*, 40, No. 4: 48. April, 1944.

A melt test, useful for determining characteristics of ice cream stabilizers is described and illustrated by photographs. In the test illustrated, 0.35% alginate was compared with 0.15% soluble soya lecithin. The latter produced a slower melting ice cream as indicated by less drainage and longer maintenance of shape. F.J.D.

240. **Injecting Pectinized Syrups, Ices or Sherbets into Ice Cream.** R. E. HAMILTON, Cleveland Ice Cream Co., Cleveland, Ohio. *Ice Cream Trade Jour.*, 40, No. 4: 30. April, 1944.

A mechanical injecting device is described for automatically incorporating various flavoring syrups, usually containing fruit or chocolate, into ice cream as it comes from a continuous freezer and moves toward the packaging point. A similar device is described for the injecting of previously frozen ices or sherbets into ice cream. The author uses a continuous freezer for pumping the material to be rippled, but notes that there is a pump on the market which should be capable of doing the job satisfactorily. F.J.D.

241. **Quality in Sherbets.** SAMUEL SABEL. *Ice Cream Trade Jour.*, 40, No. 4: 29. April, 1944.

The author issues a warning to ice cream manufacturers against a very common practice he has noted in all sections of the country during a recent business tour of the nation. The practice is that of concocting so-called sherbets from whatever materials are available when milk solids quota are exhausted merely to provide dealers with something to sell. In the author's opinion, this is a great mistake and one which not only reacts against future sherbet sales but will cause loss of confidence in the manufacturer which will carry over into the post-war period. He finds the more far-sighted manufacturers ceasing manufacture until the next period when it is not possible to manufacture to at least minimum standards. F.J.D.

242. Dried Whole Egg Powder. VIII. An Improved Fluorescence Method and Some Factors Affecting the Measurement. J. A. PEARCE, M. W. THISTLE, AND MARGARET REID, Natl. Res. Labs., Ottawa. *Canad. Jour. Res., D. 21*, No. 11: 341. 1943.

This laboratory had previously reported a method for assessing egg powder quality by determining the fluorescence of a potassium chloride extract of the defatted powder. (See *Jour. Dairy Sci. Abs.*, A220, 1943.) In order to save time and reagents the test has been modified as follows: 2.5 gm. of egg powder is defatted with three 25-ml. portions of chloroform; after drying at room temperature for about 1 hour, 1 gm. of the defatted powder is shaken for 30 minutes with 100 ml. of 10% sodium chloride solution, filtered, and the fluorescence of 15 ml. of the filtrate determined in the photofluorometer.

Increasing the temperature of the extraction raised the fluorescence values but pH changes between 4.6 and 8.9 caused no significant effect on results. O.R.I.

243. Dried Whole Egg Powder. IX. Effect of Drying Conditions on Quality. A. H. WOODCOCK AND MARGARET REID, Natl. Res. Labs., Ottawa. *Canad. Jour. Res., D. 21*, No. 12: 389. 1943.

Liquid whole egg was spray-dried in a small laboratory drier at various rates of flow of liquid egg and at different inlet and exhaust air temperatures. Quality of the powder as assessed by chemical methods, palatability and baking tests, was progressively improved as the exhaust air temperatures were lowered. Inlet air temperatures above 225° F. had a deleterious effect. Lowering the drying temperature, however, had an adverse effect on the rate of production. O.R.I.

244. Dried Whole Egg Powder. X. The Effect of Added Substances on the Keeping Quality. JESSE A. PEARCE, A. H. WOODCOCK, AND N. E. GIBBONS, Natl. Res. Labs., Ottawa. *Canad. Jour. Res., F. 22*, No. 2: 34. 1944.

Dried whole egg powders, treated with a number of substances prior to drying, were stored at temperatures from 75° F. to 118° F. Deterioration in quality was assessed by fluorescence measurements, supported in some instances by palatability tests.

Fluorescence development in powders containing sodium chloride in combination with either citric or lactic acid was more rapid than in the control powder. The effect was less marked when any of these substances was used alone. The addition of 15% sucrose was more effective in inhibiting fluorescence development at 75° F. than at 99° F. but had no effect at 118° F. The addition of 0.2% sodium bicarbonate, an amount that did not affect the flavor of the powder, retarded deterioration as indicated by fluorescence and palatability tests. Other alkaline salts studied (sodium acetate, benzoate, citrate, salicylate and tartrate) had no effect. O.R.I.

**245. Ices and Sherbets.** R. J. RAMSEY, Ramsey Labs., Cleveland, Ohio.  
Ice Cream Field, 13, No. 3: 8, 66. March, 1944.

The author gives an ice and a sherbet recipe and lists brief comments on some of the important problems related to the manufacture of ices and sherbets.

He states that 50 to 60% overrun is satisfactory for sherbets whereas 20 to 35% overrun should be maintained for ices.

He makes the following war-time recommendations regarding ices and sherbets:

1. Make at least 80% of the package ice cream as half ice cream and half sherbet or as sherbet ripple.

2. Sell as much bulk sherbet as possible.

3. Make at least 50% of the bulk gallonage as sherbet combinations—25 to 30% sherbet and 70 to 75% ice cream. W.C.C.

## MILK

**246. Wild Onion and Garlic.** L. V. SHERWOOD. Univ. of Ill. Agr. Expt. Sta. Cir. 572. 8 pages. March, 1944.

Directions on how to recognize and control these noxious weeds. Cultural control is most effective; oil sprays can be used but the cost is high.

J.G.A.

**247. Recent Developments in Milk Control.** C. K. JOHNS, Dept. of Agr., Ottawa. Canad. Jour. Pub. Health, 35, No. 1: 33. 1944.

The efficient use of the laboratory may assist greatly the farm inspection staff and reduce travelling by the use of simplified tests for milk quality. These include the sediment test, dye reduction tests, and the direct microscope clump count. The resazurin test is a particularly good aid in detect-

ing milk from unhealthy udders. For the control of pasteurized milk the phosphatase and coliform tests are now coming into general use and are tending to supplant the agar plate method. O.R.I.

248. **Women in the Milk Industry.** T. J. HAMMELL, The Borden Co., New York, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 129, 1943.

Experience with women in plants was gained in the first World War: Union approval and cooperation was secured. When the same work was done the same pay was given as to men. An endeavor was made to secure women whose husbands were in the armed forces. More time was needed to break in the women workers as they were new to such work. The women are appreciative and do good work. A.C.D.

249. **Construction of Weighing Vats a Major Factor in Accurate Butterfat Sampling.** ELVIN R. ALBEE, Dept. of Health, Albany, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 125, 1943.

A study of milk weigh vats showed several that were not conducive to securing good mixing of the milk by dumping. A long narrow weigh vat gave samples that varied 0.4% in butterfat but the condition was helped by baffle plates. In another plant with rectangular two-compartment weigh cans the mixing was not good but the poorest mixing was in a weigh can with a strainer outlet near the bottom of the weigh can. In both these cases the mixing was good when the strainers were removed. The variations in test were both for and against the dealer. A.C.D.

250. **Milk Prospects for 1944.** FRED H. SEXAUER, Dairymen's League Coop. Assoc., New York, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 103, 1943.

This statement before the New York State Joint Legislative Committee deals with prices, feed supplies, numbers of cattle, and milk consumption with special reference to the New York area. All factors indicate a reduced milk supply in 1944 and the final prospects will be affected greatly by the program of the Federal Government. A.C.D.

251. **Cleaning and Sterilizing High-Temperature Short-Time Pasteurizers.** LEWIS SHERE, The Diversey Corp., Chicago, Ill. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 91, 1943.

It has been shown that the type of material to be removed from high-temperature pasteurizers is not the same as that found in vat pasteurizers. In vat pasteurizers the material is held together and to the equipment by casein; hence it can be removed by ordinary alkaline washing powders that

dissolve casein. In high-temperature pasteurizers the casein has combined with the mineral salts and cannot be so easily removed.

The best procedure for cleaning high-temperature pasteurizers is to cool the equipment by pumping through cold water until clear, then circulating an acid-base milkstone remover at 135°–150° F. for  $\frac{1}{2}$  to 1 hour, flushing out with warm water for 10 to 15 minutes, then circulating an alkaline cleansing solution at 135°–145° F. for  $\frac{1}{2}$  to 1 hour, and finally rinsing with cold water. Dismantle and brush with alkaline cleanser, if necessary. Just before using, rinse with warm water, assemble, and sterilize. Chlorine solution is preferred for sterilizing but hot water is excellent if temperature is properly maintained throughout the equipment. A.C.D.

- 252. Trends in the Administration of the Supervision of Country Milk Supplies.** G. W. MOLYNEUX, Dept. of Health, White Plains, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 7, 1943.

Recent developments have brought the public health inspection of milk into the economics of price regulation and the restriction of trade by regulation of the scope of producing areas. These two unwarranted activities together with duplications of inspections by cities could best be handled by placing all inspection of milk for both country and city under one central state milk inspection agency. A.C.D.

- 253. Mutual Aid Between Pasteurizing Plants.** C. S. LEETE, N. Y. State Dept. Health, Albany. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 85, 1943.

As a war measure the New York State War Council as a central agency to prevent duplication and omissions set up an Office of Emergency Milk Supplies to arrange for the provision of milk to the public in case of a military disaster. The plan was based upon local and voluntary cooperation of milk distributors serving a given local area. The idea was that local cooperation would solve the emergency.

Although there have been no military disasters the plan has met emergencies satisfactorily. In one city a large milk plant burned about midnight. Milk was diverted to other plants, for one day other plants supplied customers of the burned plant, but thereafter the milk of the burned plant was processed by the other plants and delivered by the plant that had met with disaster. A.C.D.

- 254. A Northeastern States Code for Milk for Pasteurization.** WALTER D. TIEDEMAN, N. Y. State Dept. of Health, Albany, N. Y. 17th Ann. Rpt. N. Y. Assoc. Milk Sanit., p. 63, 1943.

Much progress has been made in milk safety and quality by health officials and others. However, the tendency in recent years has been to

exceed the needs for a safe milk of good flavor, appearance, and keeping quality. There is need to unify regulations, and with this thought in mind health officials of eastern United States met and drew up "Northeast States Emergency Sanitation Standards for Raw Milk for Pasteurization." A copy of the regulations is presented.      A.C.D.

- 255. Milk Plant Equipment in War-Time.** O. K. BURROWS, Cherry-Burrell Corp., Chicago, Ill. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 51, 1943.

In 1942 manufacturers of dairy equipment went into war work and for the last two years replacements have been about 15% of normal. Not until 1944 has authorization been given to increase the manufacture of some dairy equipment. In addition to metal scarcity, there is the direct competition of war machines for parts such as nine motors of dairy size used on each Flying Fortress. There will be no immediate startling new equipment when the war is over. There may be some new metals and plastics and a broader experience gained in war work.      A.C.D.

- 256. Dairy Cleansers and War Requirements.** H. W. LEHMKUHL, Milk Plant Specialties, Rochester, N. Y. 17th Ann. Rpt. of N. Y. State Assoc. Milk Sanit., p. 37, 1943.

It is pointed out that cleansers have been greatly improved in the last few years, that the war-time shortage may become acute, requiring less concentrated solutions and more brushing to obtain clean equipment.      A.C.D.

- 257. Labor in War-time for Dairy Farms and Milk Plants.** A. D. GENTLE, Manpower Service, Albany, N. Y. 17th Ann. Rpt. of N. Y. State Assoc. Milk Sanit., p. 33, 1943.

This article discusses the manpower situation as it applies to dairy work. Some details of the Selective Service regulations are given.      A.C.D.

- 258. The Effect of War-time Shortages upon Maintaining Sanitation on Dairy Farms and in Milk Plants.** PAUL CORASH, N. Y. City Dept. of Health. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 23, 1943.

The war-time shortages in milk plants and on dairy farms are discussed under three headings: manpower, supplies and equipment.      A.C.D.

- 259. War Time Increases Our Responsibilities.** MILTON R. FISHER, D.V.M., Chief, Milk Control Dept. of Public Welfare, Health Div., St. Louis, Mo. Jour. Milk Technol., 6, No. 6: 362. Nov.-Dec., 1943.



Some of the factors discussed which tend to increase inspection responsibilities are as follows:

Sudden increase in population in defense areas.

Increase in demand for milk.

Lack of new equipment to handle milk. Old equipment operated above capacity in some plants.

Greater need for sanitation.

Need to be careful about making concession that would jeopardize the dairy industry, the consumers as well as the milk sanitarians. L.H.B.

## MISCELLANEOUS

260. **Measurement of Detergency: Photometer for Determination of Films on Transparent Surfaces. Determination of Rate of Hard Water Film Formation in Washing of Glass Objects.** JOHN L. WILSON AND ELWYN E. MENDENHALL, Economics Laboratory, Inc., St. Paul, Minn. *Jour. Indus. and Engin. Chem., Analyt. Ed.*, 16, No. 4: 251, 253. April, 1944.

The calcium and magnesium salts present in hard water react with many detergents to form insoluble compounds. In processes such as commercial dishwashing, some of the precipitate formed attaches itself to the objects being washed and builds up an unsightly film. A simple inexpensive photometer has been designed for the quantitative determination of this "hard water" film. Data are presented to show the reproducibility of results obtained by the suggested method and the ease and accuracy with which differences between detergents may be determined. B.H.W.

261. **Analysis of Soap-Synthetic Detergent Mixtures in Bar Form.** DONALD BERKOWITZ AND RUBIN BERNSTEIN, Detergent Section, Test Lab. U. S. Navy Yard, Philadelphia, Pa. *Jour. Indus. and Engin. Chem., Analyt. Ed.*, 16, No. 4: 239. April, 1944.

A procedure for the analysis of commercial soap-synthetic detergent mixtures is proposed which has given sufficiently accurate and reproducible results. The method is based on the separation of active ingredients from inorganic salts by means of ethyl alcohol, and the subsequent determination of soap, fatty matter, and sodium chloride in the alcohol-soluble portion. Synthetic detergent is calculated as the difference between total alcohol-soluble matter and the sum of soap, fatty matter and alcohol-soluble sodium chloride. B.H.W.

262. **Stephen Moulton Babcock.** H. H. SOMMER, Univ. of Wis., Madison, Wis. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 77, 1943.

This brief biography of Dr. Babcock is especially clear and thorough and ought to be read in its entirety. A.C.D.



# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

### BUTTER

263. Heat Resistant Coliform Organisms with Particular Reference to Butter. H. F. LONG, T. I. HEDRICK, AND B. W. HAMMER, Iowa Agr. Expt. Sta., Ames, Iowa. Jour. Milk Technol., 7, No. 1: 20. Jan.-Feb., 1944.

Heat resistant coliform organisms were encountered in pasteurized milk, in cheese made from pasteurized milk, and in butter. Most of the studies were made on butter.

Of 116 cultures isolated from butter, 48 survived 61.7° C. for 30 minutes and 28 survived for 40 minutes. In some of the resistant cultures appreciable numbers of cells were still present after 50 minutes exposure at the above temperature. All resistant cultures were of the *Escherichia* types. No heat resistant *Aerobacter* cultures were encountered.

The authors state that "The general results suggest that *Escherichia* cultures isolated from pasteurized dairy products should be tested for heat resistance before assuming that pasteurization was inadequate or that contamination had occurred."

L.H.B.

264. Starters and Cultures. M. W. HALES, Chr. Hansen's Lab., Milwaukee, Wis. Natl. Butter and Cheese Jour., 35, No. 5: 24, May, 1944; and Natl. Butter and Cheese Jour., 35, No. 6: 34, June, 1944.

This article is a review of what the nature and characteristics of starters and starter cultures should be. The author maintains that starters are selected for their ability to produce acid, flavor and aroma under commercial conditions and can be expected to do so if they are given careful uniform treatment from day to day and are renewed frequently. He warns that liquid cultures shipped by laboratories may contain small bubbles of carbon dioxide which are harmless. He strongly recommends the use of separate mother cultures instead of transfers of inoculum from bulk starter to bulk starter.

W.V.P.

### CHEMISTRY

265. The Solubility of Water in Butterfat. C. C. THIEL, Officer of Council. Jour. Council Sci. Ind. Res. (Austral.), 16, No. 3: 139-141. Aug., 1943.

Determination of the solubility of water in butterfat over the temperature range of 40° to 95° C. showed that the solubility is 0.19 per cent at 40° C., 0.26 per cent at 60° C., 0.36 per cent at 80° C., and 0.47 per cent at 95° C.

Use of 8 per cent NaCl solution in place of water had no effect on the solubility, but mixed fatty acids from butterfat, when dissolved in fat to the

extent of two per cent, increased the solubility of water at 40° C. by 0.02 per cent. W.C.F.

266. **The Determination of Moisture in Dried Butterfat.** C. C. THIEL, Officer of Council. Jour. Council Sci. Ind. Res. (Austral.), 16, No. 3: 135-138. Aug., 1943.

A comparison of different methods for the determination of moisture in butterfat showed that entrainment with nitrogen and the Fischer titration method gave similar results, but toluene distillation gave results 0.015 per cent lower. Heating methods gave consistent results equal to or only slightly lower than those obtained by the above methods, when conditions of heating were carefully standardized to yield maximum readings. Recommended heating methods were: (a) in a hot-air oven at 103° C. for three hours, (b) on a hot plate for 1½ to 2 minutes followed by 40 minutes in a vacuum oven at 100° C., or (c) on a hot plate at 160° C. for 3-4 minutes.

The Fischer titration method is rapid and uses a relatively small sample. W.C.F.

267. **Tests for Formaldehyde in Milk.** D. W. HORN, Bryn Mawr, Pa. Vol. 4, part 1, of the Pubs. of the Wagner Free Inst. Sci. Phila. 1944.

The author presents a study of the relative merits and limits of sensitivity of five different tests for the presence of formaldehyde in milk. He furthermore presents more specific directions for making these tests where specificity is now lacking. The tests investigated were: I, sulfuric acid—ferric sulfate test; II, hydrochloric acid—ferric chloride test; III, sulfuric acid—bromine test; IV, decolorized fuchsin test; and V, phenylhydrazine hydrochloride—sodium nitro prusside test.

The most sensitive test was found to be III; yet this test is not mentioned in the A.O.A.C. "Standard Methods." The least sensitive was V; yet this test is not vitiated by the presence of judiciously added nitrite as are the others. The major weaknesses of tests I, II and III are that they give negative results if the concentration of formaldehyde is too great. Methods of getting around this difficulty and the presence of nitrite and detecting very minute amounts of the preservative are discussed, as is also an efficient numbering and indexing system for studies of this general type involving ranges of concentration. F.J.D.

## DISEASE

268. **Too Little and Too Late.** E. O. ANDERSON, Dept. of Dairy Indus., Storrs Agr. Expt. Sta., Storrs, Conn. Milk Plant Monthly, 33, No. 4: 36-38, 40, 42, 50. 1944.

The author classifies the various types of mastitis and stresses the eco-

nostic and public health importance of mastitis. Some mastitis milk may become a real public health hazard if it is not properly cooled. Increasing attention is being given today to the mastitis problem. The presence of mastitis organisms in milk should be determined as early as possible. Such tests as (a) leucocyte count; (b) examination of incubated milk for streptococci occurring in chains; (c) colonies produced on special media; (d) reactions to the Hotis test; and (e) isolation of the causative organism and identification by fermentation reactions and serological grouping, may be used. These tests are used in the Connecticut Plan for the control of bovine mastitis.

The author lists seventeen suggestions for the prevention and spread of disease. In addition, he explains the use of chemotherapy in the control of infectious mastitis, which is briefly summarized under three conditions: treatment of milking cows, dry cows and those suffering from acute mastitis.

G.M.T.

**269. Outbreak of Septic Sore Throat Due to Reconstituted Powdered Milk.** LT. RALPH F. ALLEN (MC), U.S.N., AND LT. LOUIS S. BAER (MC), U.S.N.R. *Jour. Amer. Med. Assoc.*, 124, No. 17: 1191-1193. April 22, 1944.

Here is reported what is believed to be the first epidemic of septic sore throat caused by milk from a contaminated "mechanical cow." Practically pure cultures of beta hemolytic streptococci were isolated from 155 throat cultures. Of two men assigned to work in the milk preparation room, one gave a history of having had a sore throat and a tender swollen gland in his neck two weeks before the outbreak. The epidemic subsided when the serving of milk was discontinued.

The authors make the following suggestions for the benefit of medical officers at stations or on ships using the "mechanical cow":

"1. Foremost in importance is careful instruction of enlisted personnel operating the machine and continued close supervision of their work.

"2. Sufficient men should be assigned to the job and an adequate number of 'mechanical cows' obtained to supply the needed quantity of milk without having to 'railroad it through.'

"3. A thorough breakdown of the mechanical cow is necessary daily with careful scrubbing of all parts with alkaline washing powder followed by steam sterilization for at least one minute.

"4. Weekly checks with a standard thermometer of both the pasteurizing and cooling temperatures is essential. A recording thermometer attached to the mixing tank of the mechanical cow is desirable.

"5. Particular attention should be paid to keeping milk cans clean along the seams and prompt retinning of those that rust.

"6. Careful screening and adequate ventilation are important and often overlooked items of sanitation.

"7. Drying racks for the milk cans of approved construction are easily made and should always be used. Too often cans are placed upside down on the deck.

"8. Bulkheads should be kept painted white to encourage cleanliness and expose dirt.

"9. Frequent checks on the health of all milk handlers is an important duty of the medical officer." D.P.G.

270. **Cheese Carrying Typhoid in Alberta.** ANONYMOUS. *Canad. Dairy and Ice Cream Jour.*, 23, No. 4: 40. 1944.

An outbreak of thirty-five cases of typhoid fever in southern Alberta was finally traced to cheese. Investigation indicates that the cheese became infected through the water supply. A. G. Campbell and J. Gibbard, Laboratory of Hygiene, Department of Pensions and Health, Ottawa, Canada, added *E. typhosa* to several experimental vats of milk which were manufactured by a commercial process into cheddar cheese. Under certain storage conditions they found viable *E. typhosa* after six months in all samples and for ten months or more in a majority of them. H.E.R.

## FOOD VALUE OF DAIRY PRODUCTS

271. **Influence of Incubation at 37° C. on Stability of Thiamin and Riboflavin in Cow's Milk.** BARNETT SURE AND ZENAS W. FORD, Univ. of Ark., Fayetteville, Ark. *Soc. Expt. Biol. and Med. Proc.*, 54, No. 1: 83. Oct., 1943.

Pasteurized cow's milk was used throughout the experiment. It was found that incubating cow's milk for 22 hours at 37° C. caused 46% of the thiamin to be destroyed. Under similar treatment riboflavin was entirely stable. The critical temperature for thiamin destruction appeared to be between 31 and 37° C. The factors in the milk responsible for thiamin destruction were stable to 100° C. for 10 minutes. The minerals of cow's milk appeared to be responsible for about 25% of the thiamin destroyed.

R.P.R.

272. **Comparison of the Nutritive Value of Dextrose and Casein and of the Effects Produced on Their Utilization by Thiamine.** CURT P. RICHTER AND KATHERINE K. RICE, Johns Hopkins Hosp., Baltimore, Md. *Amer. Jour. Physiol.*, 141, No. 3: 346. May, 1944.

Sixteen rats which had access to only one foodstuff, casein and water, survived on the average 33 days while 21 rats on dextrose and water sur-



vived 37 days. The rats on casein immediately became very inactive while the rats on dextrose became even more active than on a stock diet, at least for the first 20 days. The casein rats ate only approximately one-third as much food as the rats on dextrose.

When given access to thiamin hydrochloride, 14 rats on casein survived 55 days, while 12 rats on dextrose lived 73 days. The results of these experiments thus showed under the simplest conditions that thiamine plays an important part in the utilization of protein as well as of carbohydrate.

D.L.E.

273. **Food Plays "Ambassador" Role.** MRS. JULIA KIENE, Director, Westinghouse Home Economics Institute, East Pittsburgh, Pa. *Certified Milk*, 18, No. 212: 7. Dec., 1943.

American food can be used as an "Ambassador" toward guaranteeing a lasting peace. The Army will require 15 per cent of the total supply of butter and 32 per cent of canned milk. American food will be needed in relief programs in newly liberated countries in order to prevent worldwide epidemics. Food habits, while difficult to change, will have to vary as the war progresses. Items stressed are; advertising to influence every American to produce, conserve, preserve fresh foods, share, eat the right food regardless of personal preferences, substitute plentiful for scarce foods, help keep food costs down, participate in community food projects, and to cooperate.

H.G.L.

274. **Dairy Research and Human Nutrition. Parts I and II.** O. E. REED, U.S.D.A., Washington, D. C. *Certified Milk*, 18, No. 212: 3, Dec., 1943; and *Certified Milk*, 19, No. 213: 5, Jan., 1944.

See Abstract 99, *JOUR. DAIRY SCI.*, 27, No. 3: A47. 1944. H.G.L.

275. **The Biggest Bargain in Health.** MRS. JULIA KIENE, Director, Westinghouse Home Economics Institute, East Pittsburgh, Pa. *Certified Milk*, 19, No. 214: 7. Feb., 1944.

The importance of milk as a food, together with the proper handling of milk in the home, is described. Suggestions are given on how to cook with milk to prevent excessive loss of vitamin B<sub>2</sub> and how to prevent curdling when cooking milk. Several recipes using milk are given. H.G.L.

276. **Nutrition and Health.** THOMAS PARRAN, Surgeon General, U. S. Public Health Service. *Certified Milk*, 19, No. 214: 3. Feb., 1944.

In order to prevent malnutrition, it is advised that every citizen should have a down-to-earth working knowledge of modern nutrition and choose every day the food necessary for a balanced diet. H.G.L.

277. **Loss of Riboflavin in Milk Due to Sunlight.** OLAF E. STAMBERG AND D. R. THEOPHILUS, Univ. of Idaho, Moscow, Ida. *Milk Dealer*, 33, No. 8: 32-42. May, 1944.

Since it is known that exposure to light destroys riboflavin, a series of experiments was conducted to determine the effects on riboflavin content of letting bottled milk stand on doorsteps. Mixed Holstein and Jersey milk, raw, pasteurized and homogenized, was bottled in plain and colored (brown) glass bottles and in paper bottles. Recognized tests for riboflavin content were used. All bottles were stored in the dark at 42° F. before being taken outside for direct sunlight exposure. Three tables of results are given showing percentage loss of different containers and under different conditions. Approximately one-half of riboflavin in milk in plain glass bottles is lost in 2 hours. Homogenized milk, due to greater opaqueness, showed slightly lower loss. Both brown bottles and paper bottles protected milk from loss of riboflavin in ratio of 10% to 80% in 6 hours as compared with plain glass. Protection for ordinary glass bottles of milk should be provided by the housewife to insure against loss of riboflavin due to exposure to direct sunlight.

C.S.T.

### HERD MANAGEMENT

278. **To Clean Milking Machines.** EVERT WALLENFELDT, Dept. of Dairy Indus., Univ. of Wis., Madison, Wis. *Milk Plant Monthly*, 33, No. 4: 27-28. 1944.

If cleaning the milking machine is to be carried out consistently by all dairymen, the procedure must be simple. Emphasis is placed on rinsing the machine immediately after milking. Following the rinse, the machine should be taken apart enough so that brushes will reach all surfaces. Following brushing, parts should again be rinsed in lukewarm water and assembled. The teat-cup assembly should be hung on a solution rack and filled completely with 0.5 per cent lye solution. All metal parts should be dried when not in use. Previous to milking, two gallons of fresh clean 100 ppm. chlorine solution should be drawn through the machine. The machine should be inspected regularly and any faulty parts replaced. Emphasis is placed on the daily cleaning immediately after milking, if the machine is to be kept in good order.

G.M.T.

279. **How to Sell Producers on the Value of Clipping Cows.** MELVIN A. MEYER. *Milk Plant Monthly*, 33, No. 1: 31. Jan., 1944.

Clipped cows play an important role in clean milk production. The chief obstacle in the way of clipping cows is the clipper itself. These are frequently too expensive for the small dairyman to own, and a cheap hand clipper will not do the job. A solution to this problem confronting the small

dairyman would be for the dairy plant to own a few clippers which could be loaned to producers. A plant fieldman might be supplied with one clipper with which he could clip part of the cows in various communities in his milk shed as a demonstration of the value of clipping. Clipping of a cow's flank is no cure-all for unclean and unsatisfactory milk. Cleanliness yet remains a fundamental requirement in quality milk production. G.M.T.

280. **Cold Weather Care of Milking Machine Rubber Parts.** C. K. JOHNS, Div. of Bact. and Res., Sci. Serv. Dept. of Agr., Ottawa. Jour. Milk Technol., 7, No. 1: 17. Jan.-Feb., 1944.

The use of a weak lye solution for maintaining the rubber parts of milking machines in a good sanitary condition has been definitely recognized by many investigators. However, it has one drawback in that the solution will freeze during cold weather.

In a study made to note the effect of a five-minute treatment of lye after a suction rinse with cold water, it was found that the lye solution was more effective than it had been previously realized.

First, a four-week study was made as follows: After a suction rinse with cold water, the teat cup assemblies of the units in use at the Central Experimental Farm were filled with lye solution, which had been kept in a cold storage room at near freezing temperature. After five minutes, this solution was drained out, and the teat cup assemblies remained in the cold room until next milking. During this four-week trial the assemblies were not dismantled nor brushed and appeared quite clean at end of the period.

The inner surfaces of the inflations and tubes were rinsed with sterile water and bacterial counts were made on the recovered rinse water, and on the milk of the first cow milked with each unit. This was repeated three times during the four-week period. The average count on the rinse waters were 470 per ml. and 2,600 per ml. for the milk samples. Then to note the effectiveness of this five-minute treatment with lye solution, when the teat cup assemblies were held between milkings at higher than freezing temperatures, the assemblies after treatment for the next seven weeks were held at temperatures between 70° and 80° F.. As in the previous trial, they were not dismantled nor brushed during this period. Bacterial counts were made four times during this period in the same manner as previously. Results for the rinse waters averaged 550 per ml. and for the milk samples 3,500 per ml.

After these preliminary trials it was decided to try this treatment for a year with storage of teat cup assemblies at room temperature. During this time, however, the teat cup assemblies were taken apart every week or ten days as was the customary practice.

At these periodical inspections there was no evidence of fat or milk solids being present on the parts. There was a slight deposit of calcium

phosphate in some of the inflations after they had been in use several months. The monthly logarithmic average bacterial counts of the mixed night's and morning's milk (raw) taken from the pasteurizing vat for the year 1941-42 ranged from 5,768 to 21,530, while for 1942-43 they ranged from 4,477 to 20,000.

L.H.B.

## ICE CREAM

281. **The Present and Future Role of the Ice Cream Industry in Frozen Foods.** F. L. THOMPSEN, Bur. Agr. Economics, U.S.D.A., Washington, D. C. *Ice Cream Trade Jour.*, 40, No. 6: 10. June, 1944.

The author reports on a comprehensive survey of the present and potential future developments of the frozen food business in the ice cream industry. Of the total civilian pack of frozen foods in 1942, the ice cream industry distributed 5.6 per cent. Ten per cent of all retail ice cream outlets sold frozen foods, and 67 per cent of these utilized regular ice cream cabinets for holding the products. Studies indicated that ice cream manufacturers are in a particularly favorable position to embrace this additional activity, either by acting as distributors of foods frozen by another organization or by actually utilizing excess freezing space for freezing the foods themselves. Methods of operation are discussed and equipment of various types are described and evaluated. The costs will vary considerably with various factors, but estimates are mentioned which will give the ice cream manufacturer some idea of the potentialities of the enterprise. F.J.D.

282. **Research-Use of Banana in Dehydrated Form.** B. I. MASUROVSKY, Res. Editor. *Ice Cream Trade Jour.*, 40, No. 6: 38. June, 1944.

F.J.D.

283. **"Velva Fruit" Research.** B. I. MASUROVSKY, Res. Editor. *Ice Cream Trade Jour.*, 40, No. 6: 28. June, 1944.

F.J.D.

284. **Outlook on Vanilla.** RAY C. SCHLOTTERER, Vanilla Bean Assoc. of Amer., Inc. *Ice Cream Field*, 43, No. 6: 18. 1944.

The author, in discussing the Bourbon vanilla situation, points out that normally about 60 per cent of our vanilla beans come from Madagascar, but that in the past three years only two shipments have been made direct from there. Beans seized by the British from French vessels attempting to evade the blockade also have been received on five occasions during that period. One shipment of over 100 tons is expected to arrive in the United States this year, it is claimed. The French are creating, and will continue to maintain, a substantial supply of vanilla in Madagascar for the mother country, the author states.

It is estimated that this year's crop of Mexican vanilla will amount to about 275,000 pounds, and the author states that the quality is reported good. Due to drouths in Mexico this year, next year's crop is expected to be low. The author estimates it will not be more than 175,000 pounds.

Tahiti vanilla has been arriving at fairly regular intervals, and it is stated that the price there has been stabilized. The price situation as a whole, however, is moving upward due primarily to uncertainty over Bourbon arrivals. It is pointed out that, if prices advance too far, vanillin will be used as a substitute for vanilla, which will eventually cause prices to decrease. Prospects for a decrease in price in the near future are very remote, it is stated. W.C.C.

285. Tests for Quality in Egg Pulp. 1. A Preliminary Note on the Application of the Reductase Test Using Resazurin as the Indicator. W. J. SCOTT AND J. M. GILLESPIE (Div. of Food Preservation and Transport, Austral.). Jour. Council Sci. Ind. Res. (Austral.), 16, No. 1: 15-17. Feb., 1943.

The time of reduction of resazurin to a pink color afforded a reliable prediction of the bacterial content of the pulp, since results correlated with the plate count. The type of flora influenced the reduction time, but the organisms were mostly *Pseudomonas*. Two milliliters of 0.005 per cent resazurin were mixed with 10 ml. of egg pulp and incubation was at 30° C.

Data given indicated a reduction time of about 5 hours when a million bacteria per ml. were present and about 2 hours with a billion. W.C.F.

286. Report of Committee on Frozen Desserts. F. W. FABIAN, Mich. State Col., East Lansing, Mich. Jour. Milk Technol., 7, No. 1: 31. Jan.-Feb., 1944.

Although these are especially trying times, inspectors and dairymen alike should be made to appreciate the necessity of keeping dairy products sanitary. The essentials for which there should be no relaxation of vigilance are health of cows and employees; cleanliness of equipment, utensils, cows, and employees; and effective pasteurization.

It is suggested that the dairy inspector concentrate on the essentials, and let the rest go for the duration.

Reports by O. A. Ghiggoile, of California; John M. Scott, of Florida; and Andrew J. Krog, of New Jersey, are given. L.H.B.

287. Specific Gravity and Overrun in Frozen Ice Cream. P. S. LUCAS AND ROBERT E. STOUT, Dept. of Dairy Husb., Mich. Agr. Expt. Sta., Lansing, Mich. Canad. Dairy and Ice Cream Jour., 23, No. 5: 35. 1944.

A new test to determine specific gravity and overrun in ice cream.

Present formulas depend on knowing the weight or volume of mix used in making a known amount of ice cream. Such formulas are obviously impracticable for inspectors. The new method proposed permits overrun determination in a sample of frozen ice cream.

A 130-gram portion of sample was melted and poured into a tared volumetric flask. Ten grams of normal amyl alcohol sp. grav. 0.817 were added to release air from the air cells. Water was then added to make the volume to 250 cc. Flask and contents were then weighed and the weights of the contents calculated.

To illustrate:

Weight of flask and contents .....	335.75 grams
Weight of flask .....	76.75 "
Weight of contents .....	259.00 "
Weight of water (259-(130-10)) .....	119.00 "
Volume water and alcohol (119.00-12.24) .....	131.24 ml.
Volume occupied by sample (250-131.24) .....	118.76 "
Specific gravity of mix ( $130 \div 118.76$ ) .....	1.0946

Knowing the specific gravity of the mix, it is easy to calculate the per cent of overrun.

Twenty tests were made with the above method on samples of known overrun with very satisfactory results. H.E.R.

**288. Stabilizing Agents in Mix Building.** KENNETH M. RENNER, Dept. Dairy Manufactures, Tex. Technol. Col., Lubbock, Tex. South. Dairy Prod. Jour., 35, No. 4: 22. April, 1944.

At the Texas Technological College corn flour affected the flavor of ice cream less than did soybean flour or wheat flour. One per cent corn flour had an effect equal to two per cent wheat flour or three per cent milk solids not fat when added to a mix containing 8.8 per cent milk solids not fat. Two per cent corn flour caused a slick corn-starchy consistency. The use of additional vanilla flavor minimized the flour flavor. A slightly greater whipping ability resulted from the use of corn flour.

The use of vegetable flours results in an ice cream which is acceptable, but it is inferior to a high quality ice cream where the solids are derived entirely from good milk products. F.W.B.

**289. Chemical Flavor in Ices and Sherbets.** B. I. MASUROVSKY, Res. Editor. Ice Cream Trade Jour., 40, No. 5, 50. May, 1944.

F.J.D.

## MILK

290. A Comparison of the Roll-Tube and Standard Plate Methods of Making Bacterial Counts of Milk. C. C. PROUTY, H. A. BEN-DIXEN, AND S. P. SWENSON, Div. of Dairy Husb. and Div. of Agron., Wash. Agr. Expt. Sta., Pullman, Wash. Jour. Milk Technol., 7, No. 1: 5. Jan.-Feb., 1944.

The use in Europe of a roll-tube technique for the quantitative and qualitative analysis of milk and other products was observed by one of the authors. Since this technique appeared to possess certain advantages over the standard plate method, a study was undertaken for the purpose of comparing the results from the two methods.

A description illustrated by pictures of the roll-tube method is given.

Two series of comparisons between the roll-tube method and the standard plate method are given. One series was of 6 to 20 replicate tests made by each method on 14 samples of milk, while the other series was of duplicate tests made by each method on 43 samples of milk.

The counts obtained by the roll-tube method generally were lower than those obtained by the standard plate method. This was especially true in high colony count samples where the number of colonies approached 300, and probably was due to the fact that the surface area of the agar in the tubes was smaller than that of the plates, thus causing increased crowding of the colonies in the tubes.

The advantages given for the roll-tube method are that the tubes are subject to less breakage and require less storage space. They can be filled with the required amount of media, sterilized, and kept on hand for instant use. They can be readily mailed for use in the field where limited laboratory facilities are available. The tubes require only 7 ml. of agar instead of the 10-15 ml. needed for standard plates; also, less incubator space is required.

Disadvantages are—a special motor-driven apparatus is required for rotating tubes; more difficult to count colonies on tubes than on plates, especially when number of colonies approach 300; need a firmer agar (2.0%) for tube method; possibility of spreading colonies greater in tubes than in plates; tubes must be incubated with their bottom ends slightly sloping downward.

L.H.B.

291. Production of 18% Cream with Best Physical Properties. A. C. DAHLBERG, Cornell Univ., Ithaca, N. Y. Milk Dealer, 33, No. 7: 36-40. April, 1944.

A light cream, in addition to fine flavor, needs: (1) maximum viscosity, (2) no cream plug, (3) no skim milk layer, (4) no oiling off in coffee, (5) no feathering in coffee, and (6) maximum ability to color coffee. To obtain these often incompatible properties in 18% cream, the author suggests (1)

avoidance of churning or excessive agitation during transportation, (2) proper separation of high-test milk if available, (3) proper pasteurization, (4) slow cooling to increase viscosity, (5) heat treatment to increase viscosity, and (6) proper homogenization. With increased demands for milk fat in other dairy products an homogenized milk-cream mixture of 10% fat correctly labeled and sold may be a practical answer to the demand for a product more than fluid milk for use in coffee or on cereals. C.S.T.

**292. Dairy Equipment Must Be Free from Microbial Contamination.**

W. L. MALLMAN, Dept. of Bact., Mich. State Col., East Lansing, Mich. *Milk Plant Monthly*, 33, No. 2: 23-25. Feb., 1944.

In quality milk production the equipment must be physically clean and free from objectionable micro-organisms. Sanitation of equipment includes both the removal and destruction of the bacteria which may be present. In destroying micro-organisms the killing agent must either enter the organism or must come into intimate contact with its cell wall. Hence if the organism is completely covered with organic or inorganic soil, such as milk proteins, milk stone or water stone, it is practically impossible to reach the cell with any acceptable chemical or physical sanitizer. Particularly is this true in the case of milk containing thermoduric bacteria. Proper cleaning removes most of the bacteria from the utensils. Sterilizing agents are used primarily as a safety measure and assure further reduction in bacterial populations. The selection of a sterilizing agent is important, and whether heat or chemical depends largely upon the available equipment and the personnel. Heat is the most effective method of destroying microbial life. It has the advantage of penetrating through organic and inorganic matter and destroying the bacteria. However, it has the disadvantage that sufficient temperatures are not always employed, hence the use of heat may give the producer a false sense of security, particularly when thermoduric organisms are present. Chemical sterilization of dairy equipment by the use of chlorine in one of several forms should be recommended where heat is inadequate or improperly used. Chlorine has the quality of acting rapidly even in the absence of heat. Its chief disadvantage is its lack of penetrability. Like heat, chlorine is also dissipated in use. Time of exposure to chlorine is very important. When chloramine compounds are used, the exposure period should be longer to compensate for its slower action. The importance of sanitation in dairy equipment cannot be over-emphasized. G.M.T.

**293. Milk Flavor from the Producer's Standpoint.** J. L. HENDERSON, Dairy Indus., Col. of Agr., Univ. of Calif., Berkeley, Calif. *Milk Plant Monthly*, 33, No. 5: 40, 41. 1944.

By following a few basic principles, a fine-flavored milk may be produced consistently. Many feed flavors are under the control of the pro-



ducer. Feed flavor is perhaps the most common flavor defect which may be controlled by the producer. To eliminate feed flavors from the milk, feed-flavor-producing substances should not be fed during a five-hour period before milking. Salty tasting milk should be eliminated from the general supply. The development of rancidity may be prevented by mixing potentially rancid milk with normal milk in the ratio of one to four or five just before or after cooling. The major control problem of oxidized flavor lies largely with the distributor, although the producer is not free from all responsibilities. The responsibility of the producer with respect to oxidized flavor development appears to be (1) to feed and maintain the milking herd in the best possible condition, and (2) to keep the milk out of copper contact.

G.M.T.

294. **Maintaining Consumer Confidence in Milk Supplies.** KENNETH M. RENNER, Head, Dept. Dairy Manufactures, Tex. Technol. Col., Lubbock, Tex. *Milk Plant Monthly*, 33, No. 1: 36-40. Jan., 1944.

With a marked increase in consumption of milk, dairymen have been confronted with the problem of supplies. With high prices, increased labor costs and acute shortage of labor, many dairymen have considered quitting business, or securing a supply of milk not complying with the established standards. A shortage of Grade A milk has made it necessary to use milk other than Grade A. In sections where consumers have become attached to the Grade A label, it is necessary to inform them why the label was taken off the milk. People drink milk because they like it. Flavor is emphasized. Good milk is clean milk, free from sediment, produced from healthy cows, having no off-flavors and a relatively low bacteria count. All milk for resale to the consuming public should be pasteurized. Volunteer consumer rationing will assist materially in keeping the confidence of the consumer. This may be accomplished through a questionnaire to the consumer explaining the milk situation. The author lists 10 points for maintaining the milk supply. Cooperation among the various groups involved will aid materially in solving the problems of the shortage of milk supply and maintaining consumer confidence in milk.

G.M.T.

295. **Planning for Future Operations in the Market Milk Industry.** P. H. TRACY, Univ. of Ill., Urbana, Ill. *Milk Dealer*, 33, No. 8: 112-115. May, 1944.

The market milk plant of the future must reduce inefficiency in plant operation in order to survive in the post-war period of over-expanded markets, increased milk sales through stores, increased bottling by chain stores, centralization of pasteurization units and increased integration. The author lists thirty conditions contributing to greater efficiency in milk plant opera-

tion in order to reduce costs and enable the post-war milk plant to survive. The sales advantages of fresh bottled milk as compared with powdered, evaporated or reconstituted milk in the post-war period are: (1) usually superior in flavor, (2) fresher, and (3) more convenient for the housewife to use. The operator of the milk plant of the future must have good business judgment, understand labor, have a technical knowledge of business and hire a man "capable of adapting his ideas to the operation of his business." C.S.T.

296. **Testing Pasteurized Milk.** A. L. PROVEN, Harper Adams Agr. Col., Newport, Salop, AND A. ROWLANDS, Midland Agr. Col., Sutton Bornington, Loughborough. Irish Agr. and Creamery Rev., 13, No. 123: (new series) 11-15. March, 1944.

The need for bacteriological examination of pasteurized milk is primarily to determine recontamination after pasteurization and to estimate keeping quality in the hands of the consumer. Therefore, simple tests other than colony and coliform counts should be carried out to ascertain the type of organisms which are carried over or recontaminate the milk, and which influence the keeping quality of bottled pasteurized milk held at normal household temperatures. To assess their effect under wartime conditions of processing milk, the authors took a survey of over 40 plants in the Midlands and found that, both bacteriologically and in keeping qualities, bottled milk had deteriorated greatly. The possibility of utilizing some simple test to supplement complete laboratory tests was therefore studied.

Results of the methylene blue test on raw and pasteurized milk at 15.5° C. (Hiscox *et al.*, Jour. Dairy Res., 4: 105) showed that in relation to keeping quality and to degree of recontamination (Rowlands and Proven Proceeding, Soc. Agr. Bact., Eng., 29) a reduction of 1½ days or less is indicative of spoilage before use by the consumer. In the experiments conducted, pasteurized commercial samples were aged 24 hours before testing. Tables are given showing results—6 hours or less after preliminary aging of 24 hours. Similar tests after aging 24 hours were conducted with the resazurin test and results tabulated. Incubation at both 15.5° C. and 18° C. for 24 hours were used and tested at 37° C. with results deemed indicative of degree of souring and deterioration occurring during aging. Several hundred samples of different milks were tested by these two modified methods and tables published. The procedure for the modified resazurin test for control of pasteurized bottled milk, including sampling, incubation, testing and interpretation of results, is given. The use of this test is summarized as follows: (1) test not applicable to freshly pasteurized milk; (2) preliminary incubation for 24 hours at 15.5° C. or 18° C. is necessary to be indicative of recontamination and keeping quality; (3) the resazurin test made at 37° C. after incubation is suitable for plant control check in

judging efficiency of cleaning and sterilization of processing plant; and (4) the results of dye reduction tests are not related to the colony count at 37° C. and do not indicate compliance with the official standard. Fourteen references are cited. C.S.T.

**297. Fieldman's Share in Producing Quality Milk—IV. Fieldman's Care of Can Washer.** VERN P. MELHUISE. *Milk Plant Monthly*, 33, No. 1: 27–31. Jan., 1944.

In addition to receiving and discharging cans, the can washer must also execute five operations, namely: (1) rinse all milk solids from each can; (2) wash each can; (3) rinse each can with hot water at minimum temperature of 180° F.; (4) practically sterilize each can with sterile rinse water and live steam; and (5) remove steam vapors and complete the drying of each can with air. Since the fieldman must explain to the producer any shortcomings of can washing, it behooves the fieldman to assume more responsibility in the receiving room operations, particularly the can washer at the receiving plant. Thus the fieldman must be thoroughly familiar with the particular can washer at the plant and know what kind of trouble to look for and where to look for it. This should not lessen the general responsibility of can washing supervision. By using a "test can," from which a 10" strip has been removed from each side, the operator may observe the operation of each jet over which it passes. The author lists ten steps in procedure for testing the can washer, and furnishes a list of items which should be checked weekly if the can washer is to be kept in good operation. G.M.T.

**298. Fieldman's Share in Producing Quality Milk—V. Fieldman Must Sell Producers on Quality Production.** W. L. SLATTER, Instr. of Dairy Technol., Ohio State Univ., Columbus, Ohio. *Milk Plant Monthly*, 33, No. 2: 22–23. Feb., 1944.

Emphasis is placed on the fact that the dairyman himself is the most important factor in the production of high quality milk. The fieldman can do much in attaining high quality milk production by emphasizing to the producer that quality milk production is not difficult, but rather doing a few necessary things at the right time. The necessary equipment and rules for production of high quality milk are listed and discussed. G.M.T.

**299. Fieldman's Share in Producing Quality Milk—VI. Practical Aspects of Chlorine and Heat Sterilization of Dairy Equipment.** M. E. McDONALD, Bur. of Dairy Serv., Calif. Dept. Agr., Sacramento, Calif. *Milk Plant Monthly*, 33, No. 3: 30–32. March, 1944.

The author compares chlorine and heat as sterilizing agents for dairy equipment. A diagram of an oil-burning hot water and steam generator

is included. Procedures and precautions in using sterilizing agents under various conditions are given. Cleanliness, sterilization, and drying are essential requirements in preparing dairy equipment for use. G.M.T.

300. **Fieldman's Share in Producing Quality Milk—VII. Keeping the Milker Clean.** GEORGE H. HOPSON, DeLaval Separator Co. *Milk Plant Monthly*, 33, No. 4: 26-27. 1944.

The author emphasizes that the milking machine should be kept clean and never allowed to become dirty. Facilities or conditions for keeping equipment clean are especially important, and contain such items as (a) desire to keep milker clean; (b) ample quantities of cold water; (c) warm and hot water; (d) brushes; (e) lye and chlorine; and (f) dairy washing powder.

Keeping the milking machine clean involves immediate rinsing, dismantling, brushing and sterilization. Immediate rinsing is probably the most important. Sterilization may be effected through hot water at 180° F. or above, or if not available, a chlorine rinse containing 250 ppm. of chlorine may be used. G.M.T.

301. **Fieldman's Share in Producing Quality Milk—VIII.** RUSSELL R. PALMER, Chief Milk Insp., Dept. of Health, Detroit, Mich. *Milk Plant Monthly*, 33, No. 5: 52-53. 1944.

Sediment in milk consists of any material or substance that was not in the milk as it came from the udder of a healthy cow. The milk may be sampled for sediment by one of several ways, namely, by drawing the milk off the bottom of the can, collecting the last of the milk as poured from the can, or taking a sample of milk from a well-stirred can. Each method is designed and employed to determine the physical cleanliness of the milk. Sediment will not increase in amount in the milk, but the bacteria associated with it may increase in numbers. The presence of sediment in milk indicates poor sanitation, and poor methods of production and handling of milk. Inasmuch as physically clean milk improperly cooled may show a high bacteria count and, conversely, high sediment milk adequately cooled may show a low bacteria count, there is no consistent relationship between the sediment test and the bacterial content of the milk. Either high sediment or high bacteria count represent unsanitary conditions and demand immediate investigation and correction. As such, both tests are definitely useful, but not definitely related to one another in results. G.M.T.

302. **Fieldman's Share in Producing Quality Milk. IX. Filtration of Milk on the Farm.** BROR E. ANDERSON AND JAMES A. ANDERSON, Johnson and Johnson, Chicago. *Milk Plant Monthly*, 33, No. 6: 26-30. 1944.

Use of a reliable filter disc will not only remove dirt from the milk, but

a study of the used disc will indicate the needed precautions in reducing the amount of dirt falling into the milk. Important reasons for filtering milk on the farm even though the milk may be subsequently filtered at the milk plant are (a) to remove extraneous matter from the milk as soon as possible, (b) to serve as convenient farm sediment check to note the type of sediment in milk, (c) to encourage production of milk with decreased amount of sediment in it, (d) to aid in detecting mastitis, and (e) to lighten the burden on milk plant filters. A good milk filter should be fast, efficient, single-service with the fibers bonded together so they will not tear apart under pressure. Filtering of milk should be done promptly after milking while the milk is still warm. Bumping of the strainer to hasten filtering should not be tolerated. Filtration is an aid in clean milk production rather than in "cleaning" milk.

G.M.T.

303. Preventing Defects in Bottled Milk and Cream. E. L. FOUTS, Agr. Expt. Sta., Gainesville, Fla. South. Dairy Prod. Jour., 35, No. 4: 85. April, 1944.

Wartime conditions have tended to increase defects in bottled milk and cream because: (1) New supplies of milk have been necessary to supply demand; (2) It has been necessary to depend largely on inexperienced employees; (3) There is a shortage of equipment and much machinery is badly worn; (4) Limitations on transportation have necessitated less frequent delivery.

Two of the most important off flavors are oxidized flavor and rancidity. The first is usually caused by exposure of the product to unplated copper in equipment or to direct sunlight. Every effort should be made to avoid this exposure. The latter may be caused by enzymes present in the milk when it is produced or by microorganisms. This flavor defect is particularly troublesome in homogenized milk. Pasteurization before the flavor develops will usually prevent this defect.

Bacteriological problems generally are the result of careless methods in handling the milk, insufficient cooling, and failure to properly sterilize containers and equipment. Coliform organisms may indicate fecal contamination. The remedy is the prevention of the contamination by clean methods of production, the use of sufficient refrigeration, efficient pasteurization and sterilization of equipment. Poor keeping quality is associated with the same conditions which cause bacteriological problems.

The most common other defects in cream are cream plug, oiling off, feathering, and the occurrence of a skimmilk layer. Cream plug is characterized by a tough leathery mass at the top of the bottle caused by partial churning. This defect may be prevented by avoiding excessive or prolonged agitation, especially when warm, and avoiding freezing. Oiling off is closely associated with cream plug and remedy is the same.

Feathering is a heat coagulation of the protein of the cream. This condition usually can be remedied by the addition of two to six ounces of sodium citrate to 1,000 pounds of cream.

Two-stage homogenization at 1,000 plus 500 pounds pressure after pasteurization will reduce or eliminate the occurrence of a skimmilk layer at the bottom of the bottle. Whether or not the cream is homogenized, the body of the cream will be improved by an aging period of 24 hours at 35 to 40 degrees Fahrenheit before bottling. F.W.B.

**304. The Value of the Milk Serum Agglutination Test in Safeguarding Raw Milk Supplies.** H. E. BREMER, Vt. Dept. of Agr., Montpelier, Vt. Jour. Milk Technol., 7, No. 1: 26. Jan.-Feb., 1944.

In view of the fact that the use of raw milk from Bang infected cows presents an important health control measure, some quick test is desirable. The milk serum test is used for this purpose as a screen test. Samples are taken from each can of milk produced for sale as raw milk. A positive or suspicious test demands that either the herd be officially blood tested and reactors removed or that the milk be pasteurized.

A comparison was made of the milk serum and blood serum agglutination tests for Bang's Disease on 472 cows in 31 Vermont herds. In the majority of instances less than 60 days elapsed between the milk serum tests and the blood serum tests, but in a few instances they were more than 90 days apart.

The results showed that 71.8% (339 cows) of the milk serum and blood serum tests checked. 10.8% (51 cows) of the negative tests on milk were suspicious on blood. 12.1% (57 cows) of the negative tests on milk were reactors on blood. 4.4% (21 cows) of the suspicious tests on milk were reactors on blood. 0.9% (4 cows) of the reacting tests on milk were suspicious on blood.

The milk serum test, if used as a basis for determining reactors, would undoubtedly overlook some reactors. Almost invariably the blood test will give a stronger reaction than will the milk. L.H.B.

**305. The Flavour Quality of Milk.** W. H. SPROULE, Ontario Agricultural College, Guelph. Canad. Dairy and Ice Cream Jour., 23, No. 4: 46. 1944.

Emphasis should be placed on the desirable flavors of milk instead of stressing the bad flavors. Experiments by Roadhouse and Koestler, California, show that the chlorides and lactose of milk have more to do with taste than any of the other constituents of milk. Superb flavor usually accompanies a high lactose and a low chloride content. Percentages of chlorides, lactose and fat change during lactation, and cow freshening dates should be staggered wisely.

Feed flavors may be overcome by withholding feed from the cows before milking. Milk readily absorbs flavors and should be kept in a clean, well-ventilated place. Other causes of bad flavor are bacteria, physiological disturbances in the cow and chemical activity induced by sunlight, metallic salts and enzymes.

H.E.R.

- 306. Delivery Problems Today and Tomorrow.** J. N. BAUMAN, Vice-Pres. and Sales Mgr., The White Motor Co., Cleveland, Ohio. South. Dairy Prod. Jour., 35, No. 4: 56. April, 1944.

At the beginning of the war there were some 4,500,000 motor trucks in service in this country. Conservative authorities have estimated that some 300,000 vehicles are disappearing from the highways annually. Since the number of new trucks built does not exceed 25,000 annually and they are allotted on highest priority standing, there are virtually no replacements available to private operators.

The first essential in maintenance is proper lubrication to avoid wear. Adjustments or replacement of worn parts at the proper time must be made to avoid damage to other parts. A maintenance manual should be available to the mechanic so that he may properly make adjustments and repairs.

After maintenance the second most important phase of truck care is the availability of parts when and where needed. The use of a parts book to identify the parts needed facilitates the filling of orders. The trucks for which parts are ordered should also be identified by model and serial number. The driver plays a very important part in the life of a truck. He should report danger signals to the maintenance department promptly. Full use of maintenance information from the truck manufacturer is important. Delivery service must, of necessity, be restricted as much as possible without extreme effect upon sales.

War-learned lessons on economical operations should be observed in peacetime since distributing cost will play a large part in the success of an enterprise following the war.

F.W.B.

- 307. Influence of Homogenization on the Properties of Milk and Cream.** J. L. HENDERSON, Univ. of Calif., Davis, Calif. Milk Dealer, 33, No. 8: 30, 76, 78. May, 1944.

With the exception of the manufacture of butter, every branch of the dairy industry now uses homogenization. This process increases the number of fat globules, causes adsorption of protein from the milk plasma, and increases surface at the fat-liquid interface. It influences milk and cream by producing non-creaming properties; increases viscosity; and induces rancidity, oxidized and "sunshine" flavors more readily than in unhomogenized milk or cream. The claim that homogenized milk or cream tastes richer was not substantiated in this experiment. Homogenization also reduced curd tension irrespective of the original curd hardness, and possibly

increases digestibility. It produces a whiter color due to increase in the surface of the fat reflecting light and decreases protein stability. Homogenized milk will coagulate quicker than unhomogenized. The use of returned homogenized milk in ice cream and chocolate milk is probably the best means of utilization.

Directions for Babcock, U. S. Public Health Standard and Farrall index tests for homogenized milk are given. C.S.T.

## PHYSIOLOGY

308. Initiation and Maintenance of Lactation in Dairy Heifers by Hormone Administration. RALPH P. REECE, N. J. Agr. Expt. Sta., New Brunswick, N. J. Soc. Expt. Biol. and Med. Proc., 52, No. 2: 145. Feb., 1943.

A 30-month-old Holstein-Friesian heifer received a total of 163 mg. of diethylstilbestrol dipropionate and 60 mg. of testosterone propionate in 11 weeks. During the injection period there was a gradual increase in udder and teat size but at no time was the udder filled with secretion. The peak daily milk production was 27.5 lb. and in 7 months the heifer produced 4517.1 lb. A 33-month-old Jersey heifer received a total of 273 mg. of diethylstilbestrol dipropionate in 14 weeks. At first there was a slight increase in udder and teat size and then the udder appeared to become slightly filled with secretion. At the end of the injection period the udder had increased considerably in size but it was not distended with secretion. The heifer attained a peak daily production of 33.7 lb. three months after the last injection. R.P.R.

309. Differentiation between *Brucella melitensis* and *Brucella abortus* by Precipitin Reaction. P. MORALES OTERO AND A. POMALES LEBRÓN, School of Tropical Medicine, San Juan, Puerto Rico. Soc. Expt. Biol. and Med. Proc., 52, No. 3: 197. March, 1943.

The precipitin reaction was used in an attempt to develop a simple and rapid method by which strains of *Brucella melitensis* could be differentiated from those of *Brucella abortus*. *B. abortus* and *B. suis* could not be differentiated; yet they could both be differentiated from *B. melitensis*. Three *melitensis* strains failed to precipitate in 30 minutes when tested against *abortus* and *suis* sera, but one strain did. Attention is called to the importance of using proper strains, typical in all respects, for immunization. R.P.R.

310. Secretion of Radio-active Sodium in Human Milk. W. T. POMMERENKE AND P. F. HAHN, Univ. of Rochester, Rochester, N. Y. Soc. Expt. Biol. and Med. Proc., 52, No. 3: 223. March, 1943.

Four normal young women served as subjects and at the beginning of



each experiment the experimental breast was emptied with an electric pump. Each subject received a single dose of about 65 mg. of radio-active sodium, as the chloride, in orange juice. Radio-active sodium was recovered in milk within 20 minutes after its ingestion and its peak concentration was reached in about 2 hours. Subsequent secretion slowly tapered off, some of the sodium being recovered after 96 hours.

R.P.R.

311. **Lactation Activity, Chemical Composition and *in vitro* Metabolism of Rat Mammary Tissue.** MAX KLEIBER, ARTHUR H. SMITH, AND PHILLIP LEVY, Univ. of Calif., Berkeley, Calif. Soc. Expt. Biol. and Med. Proc., 53, No. 2: 94. June, 1943.

Mammary tissue of rats of the Long-Evans strain at the end of pregnancy and at the height of lactation was analyzed for dry matter and nitrogen content, and the metabolic rate of the tissue was measured *in vitro*. The water content of the mammary tissue and the protein content of the mammary dry matter increased during lactation. Lactation did not alter the metabolic rate per unit of fresh tissue; however, it increased the metabolic rate per unit of dry matter and decreased the metabolic rate per unit of nitrogen in the tissue. Additional experiments indicated a positive correlation between the intensity of lactation and the magnitude of the effect that lactation had on the composition and metabolic rate of mammary tissue.

R.P.R.

312. **Effect of environmental Stilbestrol in Shortening Prolonged Gestation in the Lactating Rat.** CHARLES K. WEICHIERT, Univ. of Cincinnati, Cincinnati, Ohio. Soc. Expt. Biol. and Med. Proc., 53, No. 2: 203. June, 1943.

One tenth to 0.5 mg. of diethylstilbestrol scattered about the cages of inseminated lactating rats suckling 9 young brought about implantation at the normal time, day 6, rather than around the 16th day as in untreated controls. The importance of isolating experimental animals that are being treated with diethylstilbestrol was pointed out.

R.P.R.

313. **Occurrence of Premature Ovulation in the Domestic Fowl Following Administration of Progesterone.** RICHARD M. FRAPS AND ABRAHAM DURY, Bur. of Anim. Indus. Soc. Expt. Biol. and Med. Proc., 52, No. 4: 346. 1943.

Crystalline progesterone was shown to be highly effective in the domestic fowl when administered under certain conditions in causing premature ovulation of normally developing ovarian follicles. Route of injection, time from injection to expected normal ovulation, injection level, and place of the follicle in the clutch sequence influenced results. First follicles of clutch

sequences were ovulated at least 6 hours prematurely in 90 to 95% of hens injected with 0.5 and 1.0 mg. of progesterone intravenously, or 1.0 to 10.0 mg. subcutaneously. Follicles other than the first of clutch sequences were ovulated by at least 7 hours prematurely in 70 to 75% of the hens injected subcutaneously at dosage levels of 0.5 to 5.0 mg. progesterone. Under identical conditions intravenous injection of progesterone was relatively ineffective.

R.P.R.

314. **Pregnancy Maintenance in Hypophysectomized-Oöphorectomized Rats Injected with Estrone and Progesterone.** WM. R. LYONS, Univ. of Calif., Berkeley, Calif. Soc. Expt. Biol. and Med. Proc., 54, No. 1: 65. Oct., 1943.

Rats of the Long-Evans strain were caged with males and those showing vaginal sperm were hypophysectomized and spayed on days 7 or 8 of pregnancy. Hormonal injections were begun immediately after operations and continued until the day before autopsy. It was found that one microgram of estrone and 3 or 4 mg. of progesterone substituted for the ovaries and pituitary during the period of pregnancy between days 7 and 11 and for the ovaries alone from day 11 until term. It was concluded that the pituitary is only necessary during the first half of pregnancy in the rat and then probably because of its gonadotropic triad, FSH, ICSH, and lactogenic hormone.

R.P.R.

315. **Influence of Fat Mobilization on Acetone Body Production. I.** ARTHUR MIRSKY, ISABELLE GRAYMAN, AND NORTON NELSON, Univ. of Cincinnati, Cincinnati, Ohio. Soc. Expt. Biol. and Med. Proc., 51, No. 3: 363. Dec., 1942.

Two groups of ducks were fasted for 3 days and then one group was injected subcutaneously with 4 mg. of diethylstilbestrol. Every third day thereafter for 15 days blood samples were drawn for the determination of the concentration of fat and total acetone bodies. The hormone administration produced a marked lipemia but did not influence the rate of acetone body production.

R.P.R.

316. **Effectiveness of Blood and Hemin for Augmentation of Pituitary Gonadotropic Extracts in the Male.** R. K. MEYER, W. H. MC-SHAN, AND L. E. CASIDA, Univ. of Wis., Madison, Wis. Soc. Expt. Biol. and Med. Proc., 52, No. 2: 78. Feb., 1943.

Augmentation of the effect of pituitary gonadotropic extracts by the addition of hemin was demonstrated in immature male rats. The testes, seminal vesicles, and prostates were significantly heavier in rats receiving the pituitary extract in conjunction with hemin than in rats receiving an

equal amount of pituitary extract alone. No augmentation was observed in immature male pigeons or immature male chicks when hemin was added to the pituitary extract in the case of chickens or whole blood in the case of pigeons. R.P.R.

317. **Hormonal Requirements for Pregnancy and Mammary Development in Hypophysectomized Rats.** WM. R. LYONS, MIRIAM E. SIMPSON, AND HEBBERT M. EVANS, Univ. of Calif., Berkeley, Calif. Soc. Expt. Biol. and Med. Proc., 52, No. 2: 134. Feb., 1943.

Sixty international units of purified lactogenic hormone and 10 international units of estrone injected daily ensured successful implantation in rats hypophysectomized and injected from the day of sperm. These two pure substances, however, in the doses used did not adequately substitute for the intact pituitary of a pregnant rat since only about one-half of the estimated number of implantations were found to have developed normally through mid-pregnancy. Seventeen out of 29 rats showed lobule-alveolar growth of the mammary glands equaling that of normal mid-pregnancy. Twenty of the 29 rats lost in body weight. R.P.R.

318. **Influence on Growth of Thyroactive Iodocasein.** MARVIN KOGER, E. P. REINEKE, AND C. W. TURNER, Univ. of Missouri, Columbia, Mo. Soc. Expt. Biol. and Med. Proc., 52, No. 3: 236. March, 1943.

Virgin mice weighing from 13 to 15 gm. at the beginning of the experiment received thyroactive iodocasein by either oral or subcutaneous administration. Their gains in body weight and in length were compared with those of non-treated mice. The treated mice gained more in body weight and length than their corresponding controls. The experimental animals gained from 16 to 23% more in weight and an average of 28% more in length than the untreated controls. R.P.R.

319. **Influence of Pregneninolone and Pregnenolone on Spermatogenesis in Hypophysectomized Adult Rats.** J. H. LEATHEM AND B. J. BRENT, Rutgers Univ. and Roche-Organon, Inc. Soc. Expt. Biol. and Med. Proc., 52, No. 4: 341. April, 1943.

Pregnenolone was found to be capable of maintaining spermatogenesis for at least 20 days after hypophysectomy. This steroid, however, did not influence the seminal vesicles since the decrease in seminal vesicle weight was similar to that observed in the hypophysectomized controls. Ten mg. of pregnenolone given in a single injection did not influence uterine weight or the adrenal X-zone of the spayed mouse. Pregneninolone exhibited androgenic activity but failed to maintain spermatogenesis in the hypophysectomized rat. R.P.R.

320. **Lactogenic Hormone Content of the AP of the Pigeon.** VICTOR HURST, JOSEPH MEITES, AND C. W. TURNER, Univ. of Missouri, Columbia, Mo. Soc. Expt. Biol. and Med. Proc., 53, No. 2: 89. June, 1943.

The lactogen content of the anterior pituitary of two types of pigeons, the common and the White King, of both sexes were compared. Pituitaries of male pigeons contained  $\frac{1}{2}$  to  $\frac{1}{3}$  as much lactogen as the female. The pituitary of the common female pigeon contained as much or more lactogen than did that of the White King. Per 100 gm. of body weight the pituitary of the White King contained only  $\frac{1}{2}$  to  $\frac{2}{3}$  as much lactogen as the pituitary of the common pigeon. R.P.R.

321. **Response of Hypophysectomized Immature Male Rats to Pregnant Mare Serum.** J. H. LEATHEM, Rutgers Univ., New Brunswick, N. J. Soc. Expt. Biol. and Med. Proc., 53, No. 2: 209. June, 1943.

Pregnant mare serum maintained testis weight and increased the seminal vesicle weight in hypophysectomized rats treated for a 5-day period. R.P.R.

322. **Limited Effects of Certain Steroid Hormones on Mammary Glands of Hypophysectomized Rats.** J. F. SMITHCORS AND S. L. LEONARD, Cornell Univ., Ithaca, N. Y. Soc. Expt. Biol. and Med. Proc., 54, No. 1: 109. Oct., 1943.

Male and female rats of the Long-Evans Strain, from 26 to 38 days of age, and weighing from 50 to 98 gm. at the time of hypophysectomy were used. Rats were either injected immediately or 4 to 8 days after operation with progesterone alone or in combination with estrogen and with desoxycorticosterone acetate. The mammary glands were stimulated with progesterone and estrogen, either alone or in combination, only when treatment was begun immediately. The effects of the combined hormones indicated a summation of the individual responses. Desoxycorticosterone did not induce mammary gland growth. R.P.R.

323. **Local Maintenance of Spermatogenesis in Hypophysectomized Rats with Low Dosages of Testosterone from Intratesticular Pellets.** SAMUEL DVOSKIN, Columbia Univ. Soc. Expt. Biol. and Med. Proc., 54, No. 1: 111. Oct., 1943.

Testosterone pellets were placed just beneath the tunica albuginea of the testis of rats of the Long-Evans strain at the time of hypophysectomy. The absorption of testosterone was slowed by mixing with cholesterol in varying proportions. Minute dosages of testosterone caused a regional maintenance of spermatogenesis. Such dosages were much less than those necessary for

complete maintenance of the sexual accessory glands. The direct action of testosterone on the testis in the maintenance of spermatogenesis was demonstrated. R.P.R.

324. **Prolongation of Pseudopregnancy by Induction of Deciduomata in the Rat.** B. H. ERSHOFF AND H. J. DEUEL, JR., Univ. South. Calif., Los Angeles, Calif. Soc. Expt. Biol. and Med. Proc., 54, No. 2: 167. Nov., 1943.

Fifty-six female rats which were running normal estrous cycles and which were 3 to 4 months of age were used for experimental purposes. The induction of pseudopregnancy followed by the induction of deciduomata resulted in prolongation of pseudopregnancy to the 22nd day. This complete pseudopregnancy occurred in the absence of fetal tissue and was associated with the presence of metrial glands, maintenance of corpora lutea, and inhibition of follicular development. Mammary gland development was similar to that seen during incomplete pseudopregnancy and after the 12th day regression occurred. R.P.R.

325. **Electron Microscope Study of Sperm.** M. R. B. BAYLOR, A. NALBANDOV, AND G. L. CLARK. Noyes Chem. Lab. and Univ. of Ill., Urbana, Ill. Soc. Expt. Biol. and Med. Proc., 54, No. 2: 229. Nov., 1943.

Semen samples were obtained from fertile bulls which were routinely used for artificial insemination. Fresh, unstained, and unfixed samples of spermatozoa were then studied under the electron microscope. These studies showed that the anterior portion of the sperm head was always enveloped by a protoplasmic cap which appeared damaged or disappeared if sperm were stained or fixed. The tail was found to end in a brush which consisted of many free and very long filaments. It also seemed likely that the axial filament consisted of a bundle of fine fibers rather than a single relatively thick thread. R.P.R.

326. **Alterations in Mammary Structure Following Adrenalectomy in the Immature Male Rat.** CHARLES F. REEDER AND S. L. LEONARD, Cornell Univ., Ithaca, N. Y. Soc. Expt. Biol. and Med. Proc., 55, No. 1: 61. Jan., 1943.

Removal of the adrenals in either normal or castrated immature male rats resulted in an increase in the number of lateral buds of the mammary glands. In some cases increased end bud growth was noted, particularly when body growth was not markedly inhibited. Estrogen injection into adrenalectomized rats caused either dilatation of these stimulated lateral buds or further increased their number above that of the injected unoperated controls. R.P.R.

## MISCELLANEOUS

327. **Insect and Rodent Control in Dairy Plants.** DWIGHT M. DeLONG, Dept. of Zool. and Ent., Ohio State Univ., Columbus, Ohio. Milk Plant Monthly, 33, No. 3: 60-61. March, 1944.

The author briefly describes effective methods of curtailing plants infested with roaches, flies, mites, dermestids, ants and rodents. G.M.T.

328. **Giving Musca the Air.** JERRY ZICH. Milk Plant Monthly, 33, No. 3: 60-61. March, 1944.

The common house fly, *Musca domestica*, not only is a nuisance but a menace to public health, and, therefore, should be excluded from all dairies. By installing a blower over doors and above other openings to dairy buildings, a strong current of air may thus be provided which serves as a barrier for flies, which otherwise would enter the building. Small diagrams are given showing the effective arrangements of such installations. G.M.T.

329. **The Most Important Factor in the Economic and Social Structure of the Nation.** CHRIS L. CHRISTENSEN. Milk Plant Monthly, 33, No. 1: 21-25. Jan., 1944.

Milk production has undergone a steady expansion for more than a decade. This expansion is wholly justified in the present light of nutritional value of milk and its products. The American consumer has never had sufficient milk or other dairy foods needed for good health. Dairying's future lies in translating this human need into an economic demand and a nutritional appreciation for the vast quantity of milk and milk products so that all efficient dairy operations may receive fair and equitable returns. Both the producer and manufacturer have certain obligations in further development of dairying. The dairyman needs to pay more attention to the economics of production in making use of abundant and high quality feed by use of alfalfa, improved strains and new varieties of grains, renovation of blue grass pastures, establishment of alfalfa-brome grass pastures, and employment of commercial nitrogen fertilizers. In addition, he must consider improved herd management and dairy breeding as well as improved dairy barns. The dairy manufacturer must effect economy in the operation of cheese factories, evaporating plants, creameries and other processing plants, as well as savings in handling and distributing of fluid milk and other dairy products. He must try to utilize total milk production, the solids of skim milk, as well as the fat. The dairy industry in general must promote research to expand our present knowledge of dairy products and must expand this basic educational program. The dairy industry, a major branch of agriculture, and therefore a major American industry, can prosper only when other industries prosper. G.M.T.

330. **The Place of the Dairy Industry in Post-War America.** CHRIS L. CHRISTENSEN, Vice-Pres., Celotex Corp. *Certified Milk*, 19, No. 215: 7. March, 1944.

See Abstract 138, *JOUR. DAIRY SCI.*, 27, No. 4: A66. 1944.

H.G.L.

331. **Forward Thinking in the Dairy Industry.** W. A. WENTWORTH, The Borden Co., New York, N. Y. *Milk Dealer*, 33, No. 7: 96-106. April, 1944.

There are two phases to consider in planning for the dairy industry—war and post-war. The problem of supplying the greatest demand for all dairy products ever experienced is the biggest war problem and will probably be the biggest early post-war problem. Charts and tables showing changes in consumption and utilization of milk for various products are included and discussed in this article. Factors to consider in both war and post-war planning in the dairy industry are: (1) the influence of national income upon the per capita consumption of different dairy products with their relative shifts dependent upon maintenance of income; (2) rehabilitation needs of Europe and the world will influence demands for dairy products and particularly fluid milk; (3) cooperation rather than competition whereby dairy products will supplement and not supplant each other should be a factor to consider; (4) a realization of proper price relationship of all dairy products to each other and to other commodities is essential to post-war growth and maintenance of all phases of dairy manufacturing; (5) the effect of Government price fixing, both ceiling and floor prices, needs to be considered in war and post-war thinking; (6) modification and unification of public health regulations whereby needless inspections can be eliminated, together with reciprocal acceptance of high-grade standards should benefit both consumers and the industry; and (7) the retention of wartime economies of procurement, processing and delivery of milk and all dairy products is vital to continued success of the dairy industry.

C.S.T.

332. **Looking Ahead with the Dairy Industry.** O. E. REED, Chief of Bur. of Dairy Indus., U.S.D.A. *Milk Dealer*, 38, No. 8: 48-50. May, 1944.

With the advent of war two things stand out: (1) the demonstrated ability of the industry to expand, and (2) the increased civilian demand for dairy products. Despite this increased demand, nutritionally as a nation we need to consume 275 quarts of milk or its equivalent per capita per year. The present average, however, is estimated at 231 quarts a year, indicating a 19% increase in consumption of dairy products to satisfy nutritional needs. Good quality of all dairy products and efficiency in their production

and distribution will be essential to supply increased consumptive demands, and both factors must be continually stressed as post-war problems. Fluid milk as such, particularly in the heavily populated northeastern section of the United States, will retain its present market preference provided its price is kept in line with competitive products. C.S.T.

333. **Postwar Outlook for the Dairy Industry.** DONALD E. HIRSCH, Farm Credit Admin. Natl. Butter and Cheese Jour., 35, No. 6: 26. June, 1944.

The federal government will probably establish price "floors" on dairy products to prevent sudden price declines. Such declines, which would be the logical effect of surpluses, may be avoided in part by education of consumers to the merits of dairy products in their numerous forms. The success or failure of dairy organizations will hinge on their ability to evaluate properly the significance of certain industrial changes and developments such as in the efficiency of utilizing milk solids, operating economies, plant flexibility, direct marketing to consumers, standards of quality for raw material and manufactured products, methods of establishing prices, artificial trade barriers and farming methods. Many organizations in industry can meet postwar problems better by reorganization, particularly cooperatives, by improving relations with patrons and customers and by sponsoring research and using the results to educate consumers. The outlook for the dairy industry is, on the whole, not unfavorable if all of its members keep informed and ready to make necessary adjustments. W.V.P.



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## ABSTRACTS OF LITERATURE

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# PUBLICATIONS AND ABSTRACTORS

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## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	Prussian Dairy Research Institute, Kiel, Germany
International Association of Ice Cream Manufacturers	State Agricultural Colleges and Experiment Stations
International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### BOOK REVIEW

334. **The Chemistry and Technology of Food and Food Products.** Edited by MORRIS B. JACOBS, published by Interscience Publishers, Inc., 215 Fourth Ave., New York, N. Y. Volume 1, XVIII, 952 pages, 79 illustrations, 218 tables, \$10.50.

This, the first of two volumes on the title subject, contains 23 chapters prepared by 21 collaborators. The subject matter is divided into two parts: (a) fundamentals dealing with aspects of food chemistry common to all foods, (b) some account of the history, statistics, definitions, standards, composition, and chemistry of these food groups. The section on fundamentals includes: physical chemistry as applied to foods, and the instruments for measuring their physico-chemical properties (38 pages); chemistry, properties and uses of carbohydrates (36), lipids (19), amino acids and proteins (72), enzymes (41), vitamins, vitagens, hormones (40), mineral matters and inorganic food adjuncts (27), coloring matters (18); digestion and fate of foodstuffs (26); food spoilage and poisoning (24). The second part of the volume contains chapters dealing with classes of food products including dairy (39), meat (35), fish (39), poultry and eggs (18), oils and fats (21), cereal grains (89), bakery foods (40), vegetables, mushrooms, nuts and fruits (46), carbohydrate and sugar foods (24), confectionery and cacao (27), coffee and tea (16), spices, flavor and condiments (22).

For the most part these latter chapters do not include the details of processing techniques, which are evidently reserved for a second volume; but they do contain excellent reviews of problems and procedures specific and peculiar to the products, which the respective authors considered significant to that particular branch of the food industry. As a result much information useful in the arts of food technology has been assembled. On the other hand, some of the material is arranged in a technical dictionary descriptive style and as a result is brief and elementary. Even though several texts have appeared in recent years in the various fields of food technology, the approach to the subject as indicated in this first volume is different, and with breadth, and should prove a valuable correlative book in food laboratories and operations. While the section on dairy products in this volume contains no information not already available in the usual dairy texts, and is somewhat brief and elementary for those engaged in dairy industries work, the scope of the material in the balance of the text and the reference to milk in connection with other products make it a valuable augmentative text also to the dairy food industry.

K.G.W.

## BACTERIOLOGY

335. **Riboflavin Requirements of Certain Lactic Acid Bacteria.** TIARESSA E. CAMPBELL AND G. J. HUCKER, N. Y. Agr. Expt. Sta., Geneva, N. Y. Food Res., 9, No. 3: 197. May-June, 1944.

Studies were made of the riboflavin requirements of a large number of lactic acid bacteria. One culture, No. 7993 (*Lactobacillus*), was encountered which responded to smaller quantities of riboflavin than No. 7496 (*L. Casei*), presently used as the assay organism. Studies indicated that this culture (7993) could be used for assaying materials too low in riboflavin to be accurately assayed with culture 7496, *i.e.*, in the range of 0.01 to 0.10 micrograms per five milliliters. F.J.D.

336. **Relative Resistance of *Eberthella typhosa* and *Escherichia coli* to Chlorine and Chloramine.** ELSIE WATTIE AND C. T. BUTTERFIELD, Dept. of Water and Sanitation Investigations, U. S. Public Health Service, Cincinnati 2, Ohio. Jour. Bact., 47, No. 5: 444-445. May, 1944. Abs. of paper presented at the 45th General Meeting, S. A. B.

At normal pH ranges, when chlorine is the disinfecting agent, approximately 60 times as much residual chlorine as chloramine is required to produce the same extent of kill as free chlorine in the same interval of time. To secure the same extent of kill with chloramine in the same concentration as free chlorine requires approximately 20 to 30 times as long an interval for chloramine. D.P.G.

337. **The Temperature Coefficient of the Bactericidal Action of Chlorine.** ADA MAE AMES AND WINSLOW WHITNEY SMITH, Univ. South. Calif., Los Angeles. Jour. Bact., 47, No. 5: 445. May, 1944. Abs. of paper presented at the 45th General Meeting, S. A. B.

More than nine times as long is required for a given dose of chlorine to disinfect water at 8° as at 40°. (The Centigrade scale is inferred.) D.P.G.

338. **A Proposed Standard Method for the Bacteriological Examination of 30-40 Mesh Edible Gelatin.** MURRAY P. HORWOOD, Mass. Inst. Technol., Boston, Mass. Jour. Bact., 47, No. 5: 436. May, 1944. Abs. of paper presented at the 45th General Meeting, S. A. B.

Following a study of methods, it is recommended that the bacteriological examination of fine ground edible gelatin be made on one per cent solutions, on tryptone glucose agar, at 35-37° C. after 48 hours. D.P.G.

339. **Heat Activation Inducing Germination in the Spores of Thermophilic Aerobic Bacteria.** HAROLD R. CURRAN AND FRED R.

EVANS, U. S. Dept. Agr., Bur. Dairy Indus., Agr. Res. Admin., Washington, D. C. Jour. Bact., 47, No. 5: 437. May, 1944. Abs. of paper presented at the 45th General Meeting, S. A. B.

Of 10 facultative thermophilic cultures isolated from spoiled commercially-canned evaporated milk, nearly all responded to preincubation heat. A large proportion of the potentially viable spores did not germinate in the absence of such treatment. The procedure consisted of seeding washed spores into various substrates and heating at 95° C. for 10 minutes; the heated and control suspensions were then subseeded into glucose nutrient-agar plates which were incubated at selected temperatures and counted after 48 hours.

The proportion of spores which responded to a preincubation heat stimulus was found to be dependent upon the amount of heat, the nature of the heating medium, the temperature at which the spores were formed, and the temperature at which they were subcultured. As heating mediums, 0.5 per cent glucose or lactose were more effective upon heat activation than were 0.5 per cent peptone, milk, glucose nutrient-agar, beef-extract (0.3 per cent), glucose nutrient-broth, distilled water, or 0.5 per cent NaCl. D.P.G.

## BUTTER

340. **The Viability of Certain Udder Infection Bacteria in Butter Made from Raw Cream.** C. S. BRYAN AND P. S. BRYAN, Mich. Agr. Expt. Sta., East Lansing, Mich. Jour. Milk Technol., 7, No. 2: 65. March-April, 1944.

A study was made to determine the length of time pathogenic organisms of the bovine type would survive in butter.

The cream for churning was obtained from the milk of a cow, one quarter of whose udder was infected with *Str. agalactiae*, one with *Str. pyogenese*, one with a non-hemolytic *Staph. aureus* and the other with a hemolytic *Staph. aureus*, by the injection of 10 ml. of a 24-hour broth culture of the proper organism. Repeated examinations of the milk were made during the time the cream was being used for experimental purposes to be certain that udder infection had been accomplished.

The cream was allowed to separate by gravity and after removing was stored in an ice box at 40° F. Each day's supply was added for one week. The cream was then further contaminated by adding 200 ml. of a 48-hour broth culture of *Brucella abortus*, and the presence of all pathogens in the cream was confirmed. The cream was then divided into two lots; one lot was pasteurized at 145° F. for 30 minutes, and the other was not. It was found that pasteurization destroyed all of the pathogens in the cream.

The raw and the pasteurized creams were again each divided into three lots. One lot of each was churned immediately, while the other two portions of each were held for six days and twelve days at 70° F. before churning.

Half of the butter of each lot of cream was salted (2.0%), and half was not. The butter was kept in a refrigerator at about 45° F., and portions were examined twice weekly for the presence of the pathogens. The results showed that there was no difference between the salted and unsalted butters in any instance. No pathogens were found in the butter made from the pasteurized cream.

*Brucellus abortus* organisms survived for four months in the raw sweet cream butter, and for three months in each of the butters made from the raw cream ripened six and twelve days before churning.

The human and animal streptococci and the hemolytic and non-hemolytic staphylococci were recovered for six months from all of the lots of butter made from raw cream.

L.H.B.

## CHEESE

341. Bacteriology of Cheese. VII. Calcium and Phosphorus Contents of Various Cheeses, Including Relationship to Bacterial Action in the Manufacturing Procedures. H. J. ZAHNRDT, C. B. LANE, AND B. W. HAMMER. Iowa State Col. Res. Bul. 325. March, 1944.

Analyses of 52 samples of Cheddar cheese made in Wisconsin, Iowa, and New York showed marked uniformity in calcium and phosphorus contents, the average being 1.14% Ca and 0.78% P on a dry basis. Analyses of 27 samples of Swiss cheese gave high contents of both Ca and P, but pasteurized milk Swiss cheese had less Ca and P than the raw milk Swiss cheese. The Ca/P ratio was very consistent for Cheddar and Swiss cheese.

The Ca and P contents of Edam cheese compared favorably with that of Swiss cheese. Blue cheese was noticeably low in minerals, for the Ca content averaged 0.51% and the P 0.54% in the dry matter. The Ca/P ratio was definitely lower. Cottage cheese contained 0.31% Ca and 0.72% P, while cream cheese averaged 0.1% Ca and 0.23% P. The Ca/P ratios were low.

The scores of the cheese were not related to Ca and P contents, a result that would be inevitable with no control of factors known to affect cheese quality.

A.C.D.

## CHEMISTRY

342. Some Antioxidant Properties of D-Iso Ascorbic Acid and Its Sodium Salt. F. J. YOURGA, W. B. ESSELEN, JR., AND C. R. FELLERS, Mass. State Col., Amherst, Mass. Food Res., 9, No. 3: 188. May-June, 1944.

The antiscorbutic activity of d-iso ascorbic acid was found to be one-twentieth of that of l-ascorbic acid (vitamin C); but the former oxidized more readily than the latter and, when in mixture, the d-iso form was prefer-

entially oxidized, leaving the l form unoxidized. It thus appears that d-iso ascorbic acid is an excellent antioxidant for l-ascorbic acid.

Sodium d-iso ascorbate had poor antioxidative powers toward l-ascorbic acid. F.J.D.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

343. **Protein-Aldehyde Plastics. Reaction of Formaldehyde with Deaminized Casein.** D. C. CARPENTER AND F. E. LOVELACE, New York State Expt. Sta., Geneva, N. Y. *Indus. and Engin. Chem., Indus. Ed.*, 36, No. 7: 680. July, 1944.

The combining ratios between formaldehyde and deaminized casein are established over a concentration range up to 6.85% formaldehyde. At any aldehyde concentration 45% as much aldehyde is bound by deaminized casein as by acid casein. The aldehyde bound by acid casein and deaminized casein agrees closely with that expected from the content of certain individual amino acids in the respective proteins. B.H.W.

344. **Metallic Substitutes for Hot-Dipped Tin Plate.** ROGER H. LUECK AND KENNETH W. BRIGHTON, American Can Co., Maywood, Ill. *Indus. and Engin. Chem., Indus. Ed.*, 36, No. 6: 532. June, 1944.

Tin conservation measures are described; and the methods of manufacture and characteristics of hot-dipped tin plate, electrolytic and Bonderized steel plate are discussed. Data on corrosion resistance of various types and combinations of coatings are given for cans packed with many fruits and vegetables and for evaporated milk. Evaporated milk was packed in cans made of electrolytic plate of 0.5 and 0.75 pounds of tin per base box which had been treated in various ways for corrosion resistance. The electrolytic plate cans packed with evaporated milk showed much less corrosion resistance than did the 1.25-pound hot-dipped plate cans which were used for control samples. B.H.W.

## DISEASE

345. **Contribution of *Streptococcus uberis* to the Plate Count of Milk.** H. W. SEELEY, JR., E. O. ANDERSON, AND W. N. PLASTRIDGE, Storrs Agr. Expt. Sta., Storrs, Conn. *Jour. Bact.*, 47, No. 5: 440-441. May, 1944. Abs. of paper presented at the 45th General Meeting, S. A. B.

The complete milking of 23 *S. uberis* infected quarters was obtained with a sterile milking unit. When a selective blood agar medium containing crystal violet, mannitol, inulin and sorbitol was used, the *S. uberis* population of the samples was found to range from 30 to 44,000 colonies with a

logarithmic average of 3,000. There was no correlation between plate counts and leucocyte counts.

Of 15 strains of *S. uberis* incubated in milk for 60 hours, all multiplied rapidly at 60°, 70° and 97° F., and 10 cultures multiplied to some extent at 50° F. The population average of all cultures decreased at 40° F. Cultures held at 40° or 50° F. did not produce measurable changes in pH.

The authors believe that the *S. uberis* content of milk immediately after it is drawn usually has little influence on the plate count of high-count milk, but may contribute a large share of the flora of low-count milk, and that cooling to a temperature below 50° F. will prevent further changes in milk quality due to *S. uberis*.  
D.P.G.

346. **A Gastroenteritis Outbreak from Food.** R. C. PERRIELLO, Health Dept., Attleboro, Mass. Jour. Milk Technol., 7, No. 2: 68. March-April, 1944.

Although evidence is not considered conclusive, it is quite probable that raw milk was the responsible food in this particular outbreak. L.H.B.

347. **Mastitis—Laboratory Tests and Their Interpretation.** J. M. FRAYER, Associate Dairy Bacteriologist, Vt. Agr. Expt. Sta., Burlington, Vt. Jour. Milk Technol., 7, No. 2: 89. March-April, 1944.

A very good discussion of the following tests is given:

Physical examination	Catalase test
Strip cut test	Chloride test
Brom Thymol-blue test	Microscopic examination
Modified Whiteside test	Hotis test

Vermont's program is to eventually set up several demonstration herds with the idea to show what can be done with sound herd management, plus a certain amount of laboratory assistance, to lower the incidence of mastitis in the Vermont dairy herds.

The tentative diagnostic procedure will be as follows: In addition to the routine use of the strip cup, physical examination of udder and brom-thymol-blue test at the stable, aseptically drawn quarter samples (or pooled udder samples) will be taken for laboratory tests.

The Hotis test will be made on all samples, and those which are either positive or suspicious will be examined microscopically and plated on either Edward's blood agar medium or on plain blood agar as an additional check.

It is hoped that this procedure will detect incipient mastitis early and thus facilitate segregation and treatment.  
L.H.B.

348. **The Streptococcal Flora of the Non-Mastitis Udder.** J. J. REID, M. A. FARRELL, E. A. KEYES, AND J. F. SHIGLEY, Pa. State Col.,



State College, Pa. Jour. Bact., 47, No. 5: 440. May, 1944. Abs. of paper presented at the 45th General Meeting, S. A. B.

Monthly examination of 1,200 lactating cattle over a period of two years revealed that at some time during the period most of the animals shed streptococci in the milk. This was demonstrated by the Breed smear of incubated, sodium azide, brilliant-green treated milk. A few animals consistently showed neither streptococci by routine tests nor clinical evidence of udder trouble.

Twenty-five of the mastitis-free animals which had never been found to be shedding streptococci were selected for special study from two herds in which mastitis had not been a problem. Forty-ml. samples were taken from each animal and the samples were centrifuged immediately. The supernatant liquid was removed, and 5 ml. of veal infusion glucose broth containing brilliant green and sodium azide were added to the sediment. After incubation for 16 hours at 37° C., smears were examined for streptococci and blood agar streaks were made. Streptococci were demonstrated in each case. Beta hemolytic streptococci of Lancefield Group D were found in the milk of all these animals, and streptococci of other Lancefield groups were demonstrated in 22 of the 25 animals. D.P.G.

## FEEDS AND FEEDING

349. **Effect of Wilting on the Fermentation of Alfalfa Silage.** R. W. STONE, J. J. REID, P. S. WILLIAMS, AND S. I. BECHDEL, Pa. State Col., State College, Pa. Jour. Bact., 47, No. 5: 441. May, 1944. Abs. of paper presented at the 45th General Meeting, S. A. B.

Seven silages, ranging in moisture content from 30 to 65 per cent, were put up with no treatment other than wilting before raking. Four silages were prepared from the same forage with ground corn and other preservatives. All fermented satisfactorily and produced a palatable product. The rate of fermentation and total bacterial count were proportional to moisture content. All wilted silages were relatively high in reducing sugar; those wilted to 50 per cent and below contained more, on a moisture-free basis, than those wilted to 65 per cent. Final pH was inversely proportional to moisture. Lactobacilli were predominant in all silages; in those containing 60 per cent or more moisture no other organisms were present in significant numbers. Below 60 per cent, yeasts were common and in two instances increased to numbers comparable to the lactobacilli. Some spore-forming rods were found in the drier silages. D.P.G.

350. **Stability of Carotene Added to Solid Carriers.** EMANUEL BICKOFF AND KENNETH T. WILLIAMS, West. Region. Res. Lab., U. S. Dept. Agr., Albany, Calif. Indus. and Engin. Chem., Indus. Ed., 36, No. 4: 320. April, 1944.

Oil solutions of carotene may be used to supplant the large quantities of

cod- and shark-liver oils formerly added to feeds as a vitamin A supplement. The effect of the carrier, the added oil (type and concentration), concentration of carotene and added antioxidants on the stability of carotene was studied. The addition of carotene in pellet form to certain dry carriers without protective measures results in very rapid loss. With protective measures, such as favorable storage conditions, the addition of certain oils and of very small amounts of antioxidants, and a reasonably low but significant concentration of carotene, the retention of carotene is greatly improved and suggests that pelleted mixtures containing extracted carotene may prove feasible as a supplementary feed. B.H.W.

## FOOD VALUE OF DAIRY PRODUCTS

351. **Vitamin A Content of Sheep's Colostrum and Milk.** G. HOWARD SATTERFIELD AND R. E. CLEGG, Univ. of N. C., Raleigh, N. C., AND ARTHUR D. HOLMES, Mass. Agr. Expt. Sta., Amherst, Mass. Food Res., 9, No. 3: 206. May-June, 1944.

The average vitamin A content of the colostrum and milk of ewes, determined colorimetrically, was found to be 10.11, 6.88, 3.98, 3.37, 2.70, 1.03 and 1.10 Lovibond units per gram, respectively, for the first seven days of lactation. During the second and third weeks it was still lower. The vitamin A content of the colostrum and early milk was slightly higher for two-year-old ewes than for older animals. F.J.D.

352. **Iron Content of Market Milk.** FRANCES A. JOHNSTON, Dept. of Ped. and Dept. of Home Econ., Univ. of Chicago, Chicago, Ill. Food Res., 9, No. 3: 212. May-June, 1944.

Sixty-one bottles of market milk from 25 companies, in 12 communities, were analyzed for iron by Stugart's thiocyanate method. The iron content ranged from 0.114 to 0.650 mg. per kg. The mean was 0.319 mg. per kg. Probably the best value to use for average market milk is 0.3 mg. per kg. This is one-seventh of the value used in current tables of food composition. F.J.D.

## ICE CREAM

353. **Effect of Various Temperatures of Storage upon Chemical and Physical Properties of Powdered Egg Yolk as Related to Its Use in Ice Cream.** P. H. TRACY, JOHN SHEURING, AND W. A. HOSKISON, Dept. Dairy Husb., Univ. of Ill., Urbana, Ill. Food Res., 9, No. 2: 126. March-April, 1944.

At high temperatures of storage (room temperatures and above) powdered egg yolk was found to decrease in pH and to lose solubility progressively. At low temperatures (40° F. and under) little effect was noted.

Bacterial populations of the powder were found to decrease more rapidly as the time and temperature of storage were increased, and the color to increase directly with the temperature of storage. The flavor also seemed to deteriorate directly with the time and temperature of storage, and the presence of milk solids in the egg-yolk powder accelerated the rate of deterioration.

Best flavored ice cream resulted when egg-yolk powder stored at low temperature (40° F. or under) was used, but even powder stored at temperatures of 90° F. for as long as 18 months, when used in quantities representing 0.5 per cent of the mix, did not have a serious flavor effect.

Time and temperature of storage of egg-yolk powder could not be related to the whipping qualities or the melting characteristics of ice cream in which the product was used. F.J.D.

354. **Studies on the Bacteriology of Stored Dried Egg Powder.** STANLEY E. HARTSELL, Dept. of Biology, Purdue Univ., Lafayette, Ind. Jour. Bact., 47, No. 5: 439. May, 1944. Abs. of paper presented at the 45th General Meeting, S. A. B.

Neither packaging nor compression influences the bacterial content of dried eggs. Higher storage temperatures decrease the total bacterial count more rapidly than lower temperatures. Yeast-water agar is superior to Standard Methods agar for obtaining total bacterial counts and for the isolation of bacteria present in stored dried egg powder, irrespective of the temperature of incubation. Higher counts were obtained when plates were incubated at 32° C. than at 37.5° C. D.P.G.

355. **The Occurrence and Possible Significance of *Salmonella* Organisms in Canadian Dried Egg Powder.** N. E. GIBBONS AND R. L. MOORE, Div. of Appl. Biol., Natl. Res. Council, Ottawa, Canada. Jour. Bact., 47, No. 5: 439-440. May, 1944. Abs. of paper presented at the 45th General Meeting, S. A. B.

The examination of 380 carlots of dried egg powder resulted in the isolation of 8 types of *Salmonella* from 28 lots. The numbers present were small, ranging from less than one per gram in 13 samples to 54 per gram in one sample. The distribution within carlots was spotty. Laboratory spray drying produced a 99.9 per cent reduction in the *Salmonella* count and a 65-80 per cent reduction in the total viable count. On storage the number decreases rapidly. *Salmonella* organisms could not be detected in cooked, reconstituted powder that had been inoculated with 8,000 to 2,000,000 organisms per gram. The authors conclude that, if reconstituted egg is properly kept and cooked, there should be little danger from powders containing as few *Salmonella* organisms as reported. D.P.G.

## MILK

356. **The Effects of Farm Cooling Methods and Transportation on the Temperature of Night's Milk.** S. ABRAHAM AND C. H. OUTWATER, City Dept. Health, New York, N. Y. Jour. Milk Technol., 7, No. 2: 78. March–April, 1944.

The temperature of the milk at the receiving platform does not necessarily give the inspector the actual temperature to which the night's milk was cooled on the farm.

A survey was made on 2233 farms in the New York City milk shed to determine (1) the actual temperature to which the night's milk was cooled prior to its being loaded on the trucks; (2) the types of cooling methods employed; (3) the relative efficiency of the various methods used; and (4) the effect of various transportation practices on the temperature of the milk at time it reaches the plants.

It was found that 53.6% of the producers had cooled their milk to below 51° F., 42.7% to between 51° and 60° F. and only 3.7% of the producers' milk exceeded 60° F., the maximum temperature permitted by the sanitary code. This was during the months of June, July, August and September.

The methods used on these farms were as follows: 45.6% had mechanical coolers; 19.3% cooled with spring water; 16.5% used ice; and 18.6% depended on pumped well water without the use of ice.

The average summer temperatures of the milk on these farms for the various types of cooling were as follows:

Mechanical coolers—42.7° F.

Ice water cooling—50.0° F.

Spring water cooling—53° F.

Pumped well water (no ice) cooling—56.5° F.

A total of 154 routes were studied; of this number only 18 vehicles carried ice for refrigerating the milk during transportation. These 18 vehicles used an average of 316.9 lbs. of ice each, travelled an average of 62.7 miles, and carried an average of 82.3 cans of milk. The average temperature of the milk at the farm was 52.7° F. and at the plant was 52.8° F. The temperature change from the farm to the plant ranged from a minus 4° to a plus 7° F. The vehicles not iced travelled an average of 26 miles and carried an average of 65.2 cans of milk. The average temperature of the milk at the farm was 47.8° F., and 52.2° F. at the plant. The temperature change from the farm to the plant ranged from a plus 1° F. to a plus 15° F.

Only one vehicle on which no ice was used was insulated, and only two vehicles on which ice was used were insulated.

L.H.B.

357. **Applying the Direct Microscopic and Swab Tests in a Milk Control Program.** N. O. GUNDERSON, Commr. Health, AND C. W. ANDER-

son, Lab. Dir., Rockford, Ill. Jour. Milk Technol., 7, No. 2: 73. March-April, 1944.

The authors conclude that the standard agar plate count has outgrown its usefulness as a milk control procedure for pasteurized milk as well as raw milk.

The direct microscopic test on both raw and pasteurized milk and the swab test for determining cleanliness of equipment offer definite advantages over the methylene-blue reduction test and the coliform test. L.H.B.

358. **Scientific Advances in the Dairy Industry (A Review of Much Current Literature).** J. H. SHRADER, Wollaston, Mass. Jour. Milk Technol., 7, No. 2: 98. March-April, 1944.

A fine review of 110 recent articles pertaining to new developments in the dairy industry. L.H.B.

359. **Relieving Labor Problems in Creameries.** C. R. ROBERTS, Sheffield Farms Co., New York, N. Y. Jour. Milk Technol., 7, No. 2: 85. March-April, 1944.

Our labor problems can be reduced by (1) using automatic machinery wherever possible, (2) job training, (3) incentive plans, (4) giving more attention to our present personnel, and (5) encouraging employee cooperation by using "suggestions" made by them. L.H.B.



# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

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## PUBLICATIONS AND ABSTRACTORS

### EDITORS

Dahle, C. D., Dahlberg, A. C., Horrall, B. E., Petersen, W. E.,  
Tracy, P. H. and Weckel, K. G.

### ABSTRACTORS

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Doan, F. J.	Glick, D. P.	Pyenson, Harry	Yale, M. W.
	Goss, E. F.		

### JOURNALS

American Butter Review	Journal of Bacteriology
American Milk Review	Journal of Biological Chemistry
American Journal of Diseases of Children	Journal of Dairy Research
American Journal of Physiology	Journal of Dairy Science
American Journal of Public Health	Journal of Endocrinology
Archives of Pediatrics	Journal of Experimental Medicine
Australian Journal of the Council for Scientific and Industrial Research	Journal of General Physiology
Biochemical Journal	Journal of Genetics
	Journal of Heredity
Canadian Dairy and Ice Cream Journal	Journal of Industrial and Engineering Chemistry
Canadian Journal of Public Health	Journal of Infectious Diseases
Canadian Journal of Research	Journal of Milk Technology
Certified Milk	Journal of Nutrition
Cornell Veterinarian	Journal of Pathology and Bacteriology
Dairy Industries	Journal of Physical Chemistry
Dairy World	Journal of Physiology
Endocrinology	Journal of Veterinary Research
Food in Canada	Lancet
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Food Manufacture	Milk Dealer
Food Research	Milk Industry
	Milk Plant Monthly
Ice and Refrigeration	National Butter and Cheese Journal
Ice Cream Field	New Zealand Journal of Science and Technology
Ice Cream Review	
Ice Cream Trade Journal	Oil and Soap
Industrial and Engineering Chemistry	Pacific Dairy Review
	Proceedings of Society of Experimental Biology and Medicine
Journal of Agricultural Research	Refrigerating Engineering
Journal of Agricultural Science	Scientific Agriculture
Journal of American Medical Association	Southern Dairy Products Journal
Journal of American Veterinary Medical Association	
Journal of Animal Science	

### SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	Prussian Dairy Research Institute, Kiel, Germany
International Association of Ice Cream Manufacturers	State Agricultural Colleges and Experiment Stations
International Association of Milk Dealers	
National Institute for Research in Dairying, Reading, England	The Royal Technical College, Copenhagen, Denmark
New York Association of Dairy and Milk Inspectors	United States Department of Agriculture



## ABSTRACTS OF LITERATURE

### BOOK REVIEWS

360. **The Microbiology of Foods.** FRED WILBUR TANNER, Univ. of Ill., Urbana, Ill. Second Ed. Published by Garrard Press, Champaign, Ill. 1196 pages. \$12.50.

The second edition of this book is completely revised and considerably enlarged. In addition to microbiology of all types of foods, the author has included general discussions on food preservation and characteristics and classification of bacteria, yeasts and molds. Other chapters are devoted to the bacteriology of water and sewage, intestinal microbiology, microbiological methods of assaying foods for vitamins and culture media.

Eight chapters covering 345 pages deal entirely with microbiology of milk and milk products. The subjects included are: Microbiology of milk, bacteriological milk analysis, pasteurization of milk, microbiology of cream and butter, microbiology of cheese, microbiology of frozen desserts, ice cream and similar products, microbiology of concentrated milks and fermented milks. Sanitation and sanitary methods in relation to microbiology and quality of milk and milk products are not very thoroughly discussed with the exception of glass milk bottles and paper milk containers. The discussions on the last two named subjects admirably sum up available information on them. The chapter on microbiology of cream and butter includes most of the newer developments in this field such as the mold mycelia work. The discussion on butter cultures is comparatively brief. The same might be said of the chapters dealing with microbiology of cheese and dried milk.

This book is intended as a reference source. Because of its detail and numerous references to original reports and because it often does not summarize or reconcile conflicting reports on certain subjects, its use as a text may be limited somewhat. Nevertheless, the author attempts to bring the reader up to date on all phases covered. Many useful methods are outlined in detail. A number of methods that are presented are perhaps obsolete and might well have been omitted. In general the books should be valuable as a reference book for instruction purposes and for any laboratories interested in any phase of microbiology of foods.

P.R.E.

361. **Advances in Enzymology. Volume 4.** F. F. NORD AND C. H. WERKMAN, Editors. Eight chapters, 330 pages, including volume index and cumulative index of volumes 1, 2, and 3. Interscience Publishers, Inc., 215 Fourth Ave., New York. \$5.50.

This is the fourth of a series of volumes containing reviews by authorities in the field of enzymology. Previous volumes have been reviewed in the JOURNAL OF DAIRY SCIENCE. The review articles are definitely tools for the

research laboratory. At least several of the review articles in this current volume are of immediate interest to dairy research workers. These include Gramicidin, Tyrocidin and Tyrothricin by Hotchkiss; The Influence of Hormones on Enzymatic Reactions by Jensen and Tennebaum; and the Absorption Spectra of Vitamins, Hormones and Enzymes by Brode. The review on the chemotherapeutic agents by Hotchkiss is quite inclusive and instructive in the light of efforts to utilize these agents in the control of mastitis. This chapter includes a discussion on chemical nature and origin, the biological properties, and the mode of action of the chemotherapeutic agents. The chapter on absorption spectra includes considerable graphic data. Other reviews in the volume include Chemical Formulation of Gene Structure and Action; The Specificity, Classification and Mechanism of Action of Glycosidases; The Transamination Reaction; Tyrosinase; Energy Transformations and the Cancer Problem. K.G.W.

## BACTERIOLOGY

### 362. Classification of the Organisms Important in Dairy Products.

IV. *Bacterium linens*. J. OSCAR ALBERT, H. F. LONG, AND B. W. HAMMER, Iowa State Col., Ames, Iowa. Iowa Agr. Expt. Sta. Res. Bul. 328, pp. 234-259. June, 1944.

The authors have developed a technic for the isolation of *Bact. linens* from various sources. A special cheese agar was used which included potassium citrate, sodium oxalate and five per cent sodium chloride; and the smeared plates were incubated in oxygen. With this procedure *Bact. linens* developed readily, and the color production was more intense than when tryptone glucose extract agar was used and the plates incubated in air.

This organism was found to be widely distributed in certain dairy products and materials about stables but was rarely found in soil.

An alkaline reaction in litmus milk and then conspicuous proteolysis were produced by *Bact. linens*. It increased the soluble nitrogen in milk on extended incubation, but the extent of proteolysis varied considerably with different strains of the bacterium. The authors also found that the amino nitrogen, the fraction soluble in trichloroacetic acid and the soluble and insoluble fractions in ethyl alcohol or phosphotungstic acid were significantly increased.

*Bact. linens* was not lipolytic. It produced a putrid condition in salted and unsalted butter at 21° C. The organism produced volatile acids from various alcohols when grown in a medium consisting of 0.3 per cent desiccated yeast extract in water. It grew at a pH of approximately 6.0 but not at a pH of approximately 5.0 in two per cent peptone solution. It also grew at a pH of approximately 9.8. The authors show that *Bact. linens* decreased rapidly in number in litmus milk in the presence of *S. lactis*. It survived

at least 4 months in litmus milk at room temperature, and when dried on filter paper it survived for at least 3 months.

*Bact. linens* tolerated 15 per cent sodium chloride in broth or skimmilk, and there was some growth in considerably higher concentrations. The authors also found that it was easily destroyed by heat and actively produced catalase.

A detailed description of *Bact. linens* is given in the bulletin. B.E.H.

## BREEDING

363. A Comparison of Inbreeding and Outbreeding in Holstein-Friesian Cattle. J. W. BARTLETT AND S. MARGOLIN. N. J. Agr. Expt. Sta. Bul. 712. 28 pages. June, 1944.

The Holstein breeding project of the New Jersey Agricultural Experiment Station was analyzed by cow families with respect to growth, production and health. The records were examined qualitatively by means of the graphic system of Bartlett *et al.* and by the use of statistical treatment. The influence of inbreeding as compared with outbreeding was considered with respect to growth, production and health of the animals. The general conclusions derived from these studies follow:

Specific cow families and sires have the facility consistently to transmit genetic factors for superior size (height at withers measure), type, percentage butterfat, milk production, and total fat. These are cited in the observations.

At present, dairy cattle can be inbred up to a coefficient of 0.30 without reducing frame size (as measured by height and heart girth). As the undesirable recessive factors are eliminated by inbreeding and selection, it should be possible eventually to increase the coefficient of inbreeding beyond 0.20 without detrimental effects, since these latter results are due to the genetic structure rather than the actual inbreeding process. All inbred animals were superior in type to outbred animals.

The total fat production was higher for the outbred than for the inbred progeny of the same sire. Outbreeding increased total fat, whereas inbreeding merely maintained it.

When the same sires were used, inbreeding raised the percentage butterfat test twice as much as outbreeding without significantly modifying milk production. In some families there was a tendency for milk production to decline as percentage fat was increased, whereas in others, milk production was maintained as the butterfat test was increased.

Outbreeding highly inbred bulls of superior qualities produced much more rapid breeding progress than inbreeding these sires. Furthermore, the rapid improvement obtained by outbreeding superior inbred sires was markedly greater than that obtained by outbreeding superior outbred sires.

The primary contribution of the inbreeding work, thus far, lies in the development of superior inbred sires. These sires have demonstrated a marked prepotency for desirable growth, type, butterfat test, and milk production; this is probably due to the homozygosity obtained by inbreeding. The marked improvement in the production record of the herd during the last decade is probably an outcome of the inbreeding program which made the development of these prepotent superior sires possible.

A short appendix to the bulletin outlines a plan for analysis of a breeding program in which consideration is given to growth, production and health.

J.G.A.

## BUTTER

364. **Use of Neutralizer.** S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., 6, No. 8: 256. Aug., 1944.

With relatively low acid cream it is best to add neutralizer to the cold cream and agitate 10 to 15 minutes before starting to heat. If neutralizer is to be added after pasteurization, it is best, with high acid cream, to reduce the acidity to 0.25 per cent before pasteurization. Cream must be neutralized to 0.15 or slightly less per cent titratable acidity to bring the pH of the butter serum within the desirable pH range of 6.8 to 7.2. It is most important that cream be neutralized to the correct acidity, and care must be exercised accordingly in weight determinations, test accuracy, and solution strength. If the excellent neutralizer, caustic soda, is used, each pound may be added to 10 pounds of water and the mixture cooled before addition to the cream, if neutralizer flavor is to be avoided.

P.S.L.

## CHEESE

365. **Comparison of Methods for Determining Fat Content of Cheese With Particular Reference to Blue Cheese.** F. J. BABEL AND F. E. NELSON, Iowa Agr. Expt. Sta., Ames, Iowa. Natl. Butter and Cheese Jour., 35, No. 9: 18. Sept., 1944.

Fat in ripening cheese is partially hydrolyzed. The Mojonnier method extracts fat from alkaline solution, and the ammonium soaps encountered when free acids are present are not extracted in the organic solvents used because they are more soluble in water. This method, the A.O.A.C. Official Method and the Babcock method were compared in analyzing young and aged Blue and Cheddar cheese. Results on 10 samples of Blue cheese show that the Mojonnier method gives distinctly and consistently lower results than the Official method, especially with aged cheese, while the Babcock method agrees better with the Official method but is usually slightly lower. Six samples of Cheddar show similar trends. There is a direct relation between

the extent of fat hydrolysis and the variations encountered between the Mojonnier and Official methods. W.V.P.

366. The Survival of *E. typhosa* in Cheddar Cheese Manufactured from Infected Raw Milk. A. G. CAMPBELL AND J. GIBBARD, Dept. of Pensions and Natl. Health, Ottawa, Canada. *Canad. Pub. Health Jour.*, 35, No. 4: 158. 1944.

A series of experiments are described in which small vats of raw milk were seeded with various cultures of *E. typhosa* at calculated rates of 1,000 to 600,000 organisms per ml. of milk. This was manufactured into Cheddar cheese, samples of which were ripened at 40–42° F. and 58–60° F. Survival was determined by plating dilutions directly on MacConkey's as well as by transferring dilutions and/or macerated cheese to tetrathionate broth as an enrichment medium.

There was a significant difference in the viability of *E. typhosa* in cheese stored at 40–42° F. compared with that at 58–60° F. Seven out of the ten cheese stored at the lower temperature retained *E. typhosa* for over 300 days whereas it appeared that at 58–60° F. most of the *E. typhosa* were dead after three months. There appeared to be no difference in the longevity of the infecting organisms as a result of the size of inoculum. However, one strain (phage type M) appeared to be more resistant than the other two studied. Titratable acidity determinations made at intervals during ripening indicated that the higher temperature hastened acidity increases. These higher acidities could not be correlated with the death of the infecting organisms however. O.R.I.

## CHEMISTRY

367. General Characteristics of the Partial Hydrolysis Products from the Action of Proteolytic Enzymes on Casein. THEODORE WINNICK, Dept. of Chem., Univ. of Idaho, Moscow, Idaho. *Jour. Biol. Chem.*, 152, No. 2. Feb., 1944.

A study was made of the partial hydrolysis products from the action on casein of the enzymes pepsin, trypsin chymotrypsin, ficin, papain, and carboxypeptidase.

Following digestions with proteases, the average non-protein molecules contained from 5 to 7 amino acid residues, with 1.5 to 4.5 per cent of the total nitrogen in the form of free amino acids. After the removal of inorganic electrolytes in electrodialysis, some additional characteristics of the digestion products were determined, including the nitrogen content, specific rotation, and average molecular weight.

The further action of carboxypeptidase on protease digests of casein was interpreted as the splitting of free acids from the ends of polypeptide chains.

The initial products from short periods of protein digestion were compared with those from prolonged protease action. The specific rotations and ratios of amino to total nitrogen of the products did not differ significantly in these two cases, in agreement with a recent speculation on the mechanism of proteolysis. A.O.C.

368. **Component Fatty Acids of Early and Mature Human Milk Fat.** A. RICHARD BALDWIN AND HERBERT E. LONGENECKER, Dept. of Chem., Univ. of Pittsburgh, Pittsburgh. *Jour. Biol. Chem.*, 154, No. 1: 255-265. June, 1944.

Three composite samples of human milk were studied. The first two were taken from 75 individuals during the first 3 days post partum, and the third was taken from two individuals during the 22nd to 43rd day of lactation. All samples were dried by the cryotherm process; and after extraction the lipids (62.1 gms. in all) were analyzed for phospholipids, volatile fatty acids, non-volatile fatty acids, and other fat constants. Fractional distillations of the methyl esters were made.

The percentage of fat increased in the mature milk and the relatively high phospholipid content of the colostrum was markedly reduced as lactation advanced. In comparing these analyses with cow's milk fat, the most interesting observation is the relatively small amounts of low molecular weight fatty acids in the human milk fat. "Although this fact has been known previously, there has been a tendency to emphasize the occurrence of these acids in cow's milk fat as having some special significance in infant feeding. These acids, however, are not present in human milk fat in sufficient quantity to make the infant's need for them credible solely on the basis of their occurrence." The human milk fat showed larger amounts of the eighteen carbon atom fatty acids than normal cow's milk fat; and yet "the octadecadienoic acid in human milk fat may not be identical with linoleic acid of ordinary seed fat, although the latter may be present in traces." A.O.C.

369. **Fluorescence Development in Various Food Products.** JESSE A. PEARCE, Natl. Res. Labs., Ottawa, Canada. *Canad. Jour. Res.*, 22, Sect. F, No. 4: 87. 1944.

Fluorescing substances developed in the following materials during storage: high protein foods, represented by dried whole milk powder, dehydrated pork, and soya flour; high carbohydrate foods, represented by dried banana flakes and dried parsnips; and a mixed foodstuff, represented by ration biscuits.

The only change occurring in stored shortenings was a decrease of fluorescing substances in hydrogenated linseed oils. Serum extracted from

rancid butter had a higher fluorescence value than serum from fresh butter. In substances containing a high proportion of fat, fluorescence values bore little relation to deterioration as assessed by peroxide oxygen determinations.

Fluorescence tests were unsatisfactory for dried milk powders and soya flour. However, they may prove useful as a measure of the quality of dehydrated pork, dried banana, dried parsnips, ration biscuits and butter. (Author's abstract.)

O.R.I.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

370. **Future of Dried Whole Milk.** P. H. TRACY, Univ. of Ill., Urbana, Ill. Amer. Milk Rev., 6, No. 8: 234. Aug., 1944.

Dried whole milk may become a competitor for fresh whole milk, especially in bread making, the ice cream industry, and in the making of cultured buttermilk and chocolate milk. To become so, it must be a better product than formerly, be more convenient to use, and be cheaper. One of its chief faults is its tendency to develop a tallowy flavor. This tendency can be reduced by fore-warming to 170° F. for 20 minutes, or 250° for one second; high concentration; low copper content; large particle size; packaging hot; low oxygen in the package; regassing with nitrogen after 24 hours; and storage at low temperatures. The use of dried whole milk in the home will require a great amount of persistent advertising.

P.S.L.

371. **The Manufacture of Crude and Technical Lactose from Cheese Whey.** B. H. WEBB AND G. A. RAMSDELL, Div. of Dairy Res., U.S.D.A., Washington, D. C. Natl. Butter and Cheese Jour., 35, No. 7: 18, July, 1944; and 35, No. 8: 18, Aug., 1944.

Crude lactose contains at least 2% more protein-plus-ash than U.S.P. specifications permit; technical lactose is somewhat better; both must be refined to make U.S.P. grade. Crude and technical lactose can be used for human food products. Profitable manufacture requires at least 75,000 lbs. of sweet whey per day. Equipment must include vacuum pan, filter press and sugar centrifuge.

Lactose is separated from whey by clarification, concentration, crystallization and centrifuging. Proper clarification is essential to obtain a low protein and ash content in the sugar; this can be accomplished with calcium chloride, lime and hydrochloric acid, but about one-third of the original nitrogen remains in the treated whey. Concentration causes the precipitation of the insoluble salts and nitrogenous material; this requires reclarification by filtration during concentration—whey from rennet curd is particularly troublesome in this respect. The resulting lactose often contains foam-producing impurities which can be removed by recrystallizing. When 10,000 parts of clarified whey is treated with one part of the enzyme trypsin

at 130° F. and at pH 6.2 to 6.4 for one to two hours, a non-foaming sugar results after one crystallization.

Six processes for making lactose from cheese whey are briefly described.

(1) The simplified crude process concentrates whey to 55 to 60% solids, crystallizes the lactose and centrifuges away the mother liquor. This product is high in whey solids. (2) The soluble albumin process concentrates neutralized whey to 62% solids, holds the concentrate at 32° to 38° for 18 hours and then centrifuges out the lactose. (3) The sweetened condensed whey process concentrates whey sweetened with about 6% sucrose to 70% total solids, holds the concentrate overnight, centrifuges out 1 to 2 lbs. lactose per cwt. whey, leaving the mother liquor for use in sweetened foods. (4) The simplified technical process clarifies the whey by heat and acid, siphons off and concentrates the clear whey to 34° Baumé at 120° F., crystallizes the lactose slowly and removes it by centrifuging. (5) The technical process clarifies whey as in process 4, siphons or filters off the whey, concentrates it to 20° Baumé (30% lactose) decolorizes with carbon, heats the sirup to boiling, cools overnight, neutralizes to pH 6.3, boils again, filters, concentrates to 38 to 40° Baumé, centrifuges out lactose, washes and dries crystals. (6) The trypsin process clarifies whey as in process 4, neutralizes to pH 6.3, boils, filters, adds one pound of trypsin to 10,000 lbs. whey, holds one hour at 132°–138° F., concentrates to 20° Baumé and then finishes as in process 5. This process can be used with cottage cheese or casein whey.

W.V.P.

## FEEDS AND FEEDING

372. **Determination of Carotene in Dehydrated Alfalfa. A Simplified Method.** RALPH E. SILKER, W. G. SCHRENK, AND H. H. KING, Kans. Agr. Expt. Sta., Dehydration Lab., Manhattan, Kans. Jour. Indus. and Engin. Chem., Analyt. Ed., 16, No. 8: 513. Aug., 1944.

A simplified method, which avoids refluxing and phasic separations, was developed for the determination of carotene in dehydrated alfalfa. Finely ground alfalfa was allowed to stand for 16 to 18 hours in a mixture of acetone and Skellysolve B, the extract was concentrated on a steam bath to remove the acetone, the pigments were adsorbed by drawing the mixture through a magnesia column, and the carotene then eluted with a 4% solution of acetone in Skellysolve B. The solution of carotene was made up to volume and analyzed with a Beckman spectrophotometer.

B.H.W.

373. **Comparison of Molasses-Oat Silage and Phosphoric Acid-Oat Silage as Feeds for the Milking Cow.** W. A. KING. N. J. Agr. Expt. Sta. Bul. 708. 22 pages. March, 1944.

Results are reported of feeding, digestion, and N, Ca, and P balance trials



on these two types of silage with 21 cows over a period of four months during the winter of 1941-42.

Molasses-oat silage proved to be superior to phosphoric acid-oat silage in a number of ways: in phosphorus retention, in palatability, in economy of milk production, and in absence of physiological disturbances.

The oat silages were not so palatable as good corn silage; but they proved to be as good, if not better in the case of the molasses-oat silage, in economy of milk production.

There were no great differences between the digestibility of the two oat silages. The coefficient of digestibility of the crude protein was definitely higher in the phosphoric acid silage. At the same time an increased nitrogen excretion in the urine of these cows was evident, indicating that this extra digested protein was not of great value for anabolic purposes.

The coefficients of digestibility of the two oat silages when fed alone were considerably higher than when the silages were supplemented with hay and grain.

The phosphorus balance of the cows fed the phosphoric acid-oat silage with limestone showed a negative balance of 18.9 gm. daily, whereas those fed the molasses-oat silage rations and the phosphoric acid silage supplemented with hay and grain were in a positive balance. Only the group fed the molasses-oat silage alone showed a negative calcium balance. The other groups of cows received calcium supplements. J.G.A.

**374. Effect of Feeding Silages on Certain Properties of Milk: I. Effect on the Yellow Color and Flavor of Winter Milk. II. Influence on the Vitamin A Content and Distribution of Carotenoid Pigments in Milk Fat. III. Effect of Various Storage Conditions on the Stability of Carotenoid Pigments in Butter.** O. F. GARRETT AND D. K. BOSSHARDT. N. J. Agr. Expt. Sta. Bul. 710. 30 pages. March, 1944.

This study extended over three feeding seasons—1940-41 through 1942-43. The three kinds of silage studied were made respectively from alfalfa, green oats, and soybeans. All were compared with corn silage as a control. Preservatives used were molasses and phosphoric acid for the alfalfa and oat silages, and molasses and corn meal for the soybean silage. Nine Holstein cows were used in each trial, three in the control group which received corn silage and three each in the other two groups.

Conclusions on the three aspects of the work were:

- I. Alfalfa silage, whether preserved with molasses or with phosphoric acid, is capable of producing milk with higher yellow color, better flavor, and greater resistance to the development of oxidized flavor than that produced with corn silage, whereas green oat silage and soy-

bean silage are about equivalent to corn silage with respect to these factors.

- II. Eight bands, or zones, of carotenoid pigments were chromatographically isolated from corn silage, molasses-soybean silage, and corn-meal-soybean silage, and from the milk fats produced on these roughages. Only seven bands were found in pasture grass and the milk fat produced therefrom.

The observation of others that the cow is capable of selecting beta-carotene from among the various plant carotenoids and concentrating it in the milk fat is verified by the results of these studies.

Milk produced on soybean silages contained approximately the same amount of total carotenoid pigment, carotene, and vitamin A as milk produced on corn silage.

The concentration of total vitamin A in Holstein milk fat was approximately the same as that of Guernsey milk fat when the feed was the same for the two groups. Guernsey milk, however, contained more total vitamin A per quart than Holstein milk under the same conditions of feeding. The Holsteins used in this experiment produced about twice as much true vitamin A and about 25 per cent more total vitamin A but only about one-half as much carotene per day as the Guernseys.

There was some tendency for true vitamin A to vary directly with the carotene in the Holstein milk, but no such relationship was found in the Guernsey milk.

A high correlation was found between lactochrometer readings and the concentration of total pigment in milk, which justifies the use of the lactochrometer as an instrument for measuring the yellow color of milk.

- III. The carotenoid pigments of milk fat are not entirely stable during storage, even when no known contamination with copper occurs.

Alpha-carotene is the least stable and beta-carotene the most stable of the definitely identified pigments.

The presence of copper accelerates the destruction of beta-carotene but has little influence on alpha-carotene and xanthophyll.

There is a slight tendency for manganous ions to inhibit the catalytic effect of cupric ions.

Hydroquinone exhibits a definite retardation of the oxidative degradation of beta-carotene both in the presence and in the absence of added cupric ions.

There appears to be a progressive formation and destruction of the pigments in bands IIIa, IIIb, and IV. Undoubtedly these pigments are degradation products of alpha- and beta-carotene, since they fall in the chromatogram above the carotenes and below the xanthophyll group, thus indicating a larger amount of oxygen in the molecule. Their stronger affinity for the adsorbent is evidence of this fact.

J.G.A.

375. **Riboflavin Content of Milk Products for Feeding Purposes.** E. V. EVANS, D. M. YOUNG, AND H. D. BRANION, Ontario Res. Found., Toronto and Ontario Agr. Col., Guelph, Canada. *Sci. Agr.*, 24, No. 11: 510. 1944.

Microbiological assays were conducted on 58 samples of buttermilk powder, 35 samples of skimmilk powder and 9 samples of whey powder. All were of the type used in poultry and hog concentrates. Wide variations in the riboflavin content of similar products were found. The values for buttermilk powder ranged from 22 to 47 micrograms per gram, while the range was 17-23 in the case of skimmilk powder and 14-24 for the whey powder.

O.R.I.

## FOOD VALUE OF DAIRY PRODUCTS

376. **For the Greatest Common Good.** HUGO SOMMER, Univ. of Wis., Madison, Wis. *Natl. Butter and Cheese Jour.*, 35, No. 8: 17, Aug., 1944; and 35, No. 9: 56, Sept., 1944.

Our opposition to repeal of the federal tax on yellow margarine is not based on the question of nutritive equivalency. Such equivalency we do not concede because there are still unanswered questions in this field. We oppose repeal for these reasons: (1) Colored margarine would be sold as butter, the incentive being that oleo fats cost only one-fifth to one-fourth as much as milk fat. (2) Prices of all dairy products would be depressed. (3) Changes in farm practices would decrease soil fertility; even the advocates of oleo recognize that increasing field crops at the expense of livestock involves soil mining and erosion. (4) Decreased use of milk solids would be a loss in terms of human health and this despite the increased use of oleo by low-income groups which would follow repeal of the tax and coloring of oleo. The depression in prices of dairy products would reduce production of non-fat dry milk solids, a by-product which is considered essential as a bread ingredient to provide essential minerals and vitamins. Bread is a staple of the low-income-group diet. We cannot at the same time expand the use of oleo and still expect to expand the enrichment of bread with milk solids. (5) A dangerous precedent would be set in the field of imitation of food products. Actually synthetic edible fats made from petroleum

paraffin are now being made in Europe and might be introduced here with embarrassing results. Filled milk, filled cheese and filled ice cream become definite possibilities with repeal of the tax. (6) The common good of consumers, dairy farmers and producers of oil crops would all be adversely affected by repeal of the tax on colored oleo. The consumer would get less protection against fraud. Even if oleomargarine largely displaced butter, the gain in oil values could not possibly approach the loss to the dairy industry. Loss of a substantial part of the butter market would cause decided losses to the whole industry and millions of dairy farmers since the price structure of dairy products is based on the butter market. W.V.P.

## HERD MANAGEMENT

377. **Better Crop and Herd Management on New Jersey Dairy Farms.**  
G. E. TAYLOR, H. R. COX, G. H. AHLGREN, AND F. E. BEAR. N. J.  
Agr. Ext. Serv. Bul. 239. 12 pages. March, 1944.

This bulletin is based on the premise that "New Jersey dairymen can best safeguard their position in the feed situation by growing more and better quality roughage." It contains numerous specific recommendations on soil management, the growing of hay, pasture, and silage crops, and on dairy cattle feeding and management. J.G.A.

## ICE CREAM

378. **The Relative Sweetnesses of Certain Sugars, Mixture of Sugars, and Glycerol.** A. T. CAMERON, Dept. of Biochem., Univ. of Manitoba, Winnipeg, Canada. *Canad. Jour. Res.*, 22, Sect. E, No. 3: 45. 1944.

The relative sweetnesses of the sugars glucose and lactose and of the alcohol glycerol, as compared with sucrose, vary with concentration.

The relative sweetness of glucose or lactose or glycerol, compared with either of the other two, does not change with concentration, within the limits of the experimental method used.

The following have been found to be equally sweet:

- (i) 2.0% sucrose and 3.8% glucose; 10.0% sucrose and 14.7% glucose; 20.0% sucrose and 25.0% glucose.
- (ii) 2.0% sucrose and 6.5% lactose; 5.0% sucrose and 14.9% lactose; 8.0% sucrose and 21.7% lactose.
- (iii) 2.25% sucrose and 4.0% glycerol; 5.5% sucrose and 8.0% glycerol; 8.9% sucrose and 12.0% glycerol.

A method is described for calculating the sweetness of a mixture of sugars of known composition in terms of that of a specific concentration of sucrose or of glucose. (From author's abstract.) O.R.I.

379. **Microbiological Aspects of Egg Powder.** C. K. JOHNS, Dept. of Agr., Ottawa, Canada. Sci. Agr., 24, No. 8: 373. 1944.

The standards and procedure employed in the bacteriological control of Canadian dried eggs for export to Britain are described. During 1943, out of a total of 235 carlots of Grade A powder examined, over 95% gave counts of under 500,000 per gram.

As a simple plant test for the bacterial content of the melange, the Burri slant method has been found most useful. Methylene blue and resazurin tests were not so satisfactory because of end-point difficulties and the long incubation periods required. The direct microscope count was found to reveal the past history of the product more satisfactorily than did the plate count, coliform or *E. coli* test. Studies on the flora of the melange and powder revealed that the Gram negative rods which predominated in the former were generally replaced by a weak-acid-producing streptococcus in the powder.

Details are given of the methods used for bacteriological analysis. After suitable preparation and reconstitution, dilutions of the powder are plated on tryptone glucose extract agar containing 1% skim milk, the plates being incubated at 37° C. for 48 hours. For the detection of *E. coli* 2% brilliant green bile broth and eosin-methylene blue agar plates are used. O.R.I.

## MILK

380. **The Pasteurization of Milk.** G. S. WILSON, Univ. of London, School of Hygiene and Tropical Medicine. Foreword by Sir Wilson Jameson. Published by Edward Arnold & Co., London. 212 pages. 18s.

In this book a review of the evidence relating to pasteurization has been made, dealing particularly with the public health and nutritional aspects of the process. The literature reviewed is extensive, with special emphasis directed to indict raw milk as a source of human disease. The experience of England and Scotland with reference to milk-borne diseases is fully described. The efficacy with which pasteurization has controlled diseases of these types in certain communities is also cited.

The book is not a manual for the milk plant operator or laboratory worker. Descriptions are given of the holder, HTST, high (flash), and Stassano methods of pasteurization although the apparatus used is not described. The laboratory control of pasteurization is critically reviewed, particular attention being paid to the importance and limitations of the phosphatase test. A very strict standard for this test is suggested, and the advice is given that it should at times be supplemented with the guinea-pig test for tubercle bacilli.

The nutritional aspects of pasteurization are discussed from the point of view of minerals, proteins, vitamins and the growth-promoting and fertility-sustaining power. The work dealing with the vitamins of the B group is somewhat incomplete, only Vitamins B<sub>1</sub> and B<sub>2</sub> being mentioned. A chapter and an appendix are devoted to refuting some of the standard arguments that are occasionally raised against pasteurization. O.R.I.

381. Meeting New York Milk Demands. LELAND SPENCER, Cornell Univ., Ithaca, N. Y. Amer. Milk Rev., 6, No. 4: 106-108, 116-119. April, 1944.

Much milk handled by Atlantic seaboard dealers the past year has been purchased outside its normal shed and has been sold at a considerable loss. New York City escaped this condition because it maintained a potential number of inspected farms to meet peak demands. This reserve supply was greatly changed by its diversion to shortage areas during November, which, with 12.6% increase in market milk consumption, caused a shrinkage to one-half of manufactured products. New York City's demand for milk in 1944 will probably be the same as for 1943, and the same will probably be true for diversion of milk from its milk shed for November 1944. Production prospects are poorer than for 1943. Government aid can function in three ways: (1) assurance of as abundant feed as possible, (2) recognition of the necessity of labor and labor-saving equipment for the dairyman, and (3) maintenance of a price high enough to encourage dairymen to stay in business at their present size. P.S.L.

382. Factors Affecting Milk Supply in Akron, Canton, Dayton, and Portsmouth, Ohio. C. G. MCBRIDE AND R. W. SHERMAN. Ohio Agr. Expt. Sta. Bul. 652. July, 1944.

The five years 1939-1943 are included in all the markets studied; and in two markets, records for a longer period were available. The years preceding 1939 had been a period of increasing total shipments of milk to these markets. All markets were operating then under some form of base and surplus plan, and there had been a trend toward less seasonal variation in shipments.

Soon after 1939 the supply situation began to change, due to shifting of population for employment in defense plants. Of the four markets, Dayton and Akron experienced the greatest increase in demand for milk. By the fall of 1942 these markets had demand in excess of total farm shipments, and in 1943 the shortage was still greater.

Changes in market plans were made that are significant. As city sales increased, desire developed on the part of producers to do away with the base and surplus plan and thus to relieve the shipper of the necessity of taking a lower price for his "surplus" milk. One after another, beginning with Dayton and ending with Portsmouth, the markets abandoned the base

and surplus plans. Dayton introduced a quantity bonus plan to induce more shipments throughout the year. From 1939 through 1943 there has been an increase in average shipments per day, but seasonal variation of shipments has increased to a marked degree. This is a matter of real concern, because wide seasonal variation adds to the difficulties and the costs of the market milk business.

The period of withdrawals of producers in largest numbers came in 1941 and 1942. The most important reason for withdrawal from all these markets was retirement, including illness and death. Increasing demand did not turn the tide of retirement, but it appeared to induce those who remained in the market to increase their volume of shipments.

The producers whose average daily shipments were much larger than the average of the market did not hold their relative position. The reason given was the difficulty of maintaining an adequate labor force. Those with shipments below the average were more inclined to withdraw by retirement than were those in the middle brackets. The increase in shipments, therefore, came as a result of larger average shipments from farms with medium size of herd.

With dealers' sales limited by War Food Order 79, it would appear that these cities will not have great difficulty with milk supply for about 9 months of the year. Several factors work together to make fall production relatively lower, and this may create some tight supply situations in the fall months. A return to some form of base and surplus plan holds the greatest promise for solving this problem.

H.P.

**383. Cleaning Method Suggestions.** S. T. COULTER, Univ. of Minn., St. Paul, Minn. *Amer. Butter Rev.*, 6, No. 4: 118-119. April, 1944.

For washing of metal equipment where a rather hard water only is available, the author has found satisfactory a mixture of 45 pounds tetrasodium pyrophosphate, 25 pounds sodium metasilicate, 25 pounds trisodium phosphate, and 5 pounds of a suitable wetting agent as Nacconol N.R. Drefl, Swerl, M.P. 189, Vel, Neosuds, etc. The very hard water for which the above was recommended contained 24 grains of hardness per gallon, 16 grains methyl orange alkalinity, and 5 grains of sulfates. The mixture is to be used at the rate of one-fourth to one-half ounce per gallon of water, or better by using one-half gallon of a stock solution per 50 gallons of water, the stock solution being prepared by dissolving 15 pounds of the dry mixture in ten gallons of water. If the wash water in the plant is softer than the example cited, less of the sequestering agent, tetrasodium pyrophosphate, may be used in the mixture. The mixture will not remove milk stone and water stone already formed, but it does not encourage their formation as does soda ash and trisodium phosphate.

For preventing milk stone formation in farm dairy utensils, the recommendation is made that a definite procedure be followed of rinsing the

utensil in lukewarm rather than cold water both before and after washing. A chlorine rinse should be given just before use only, since chlorine solution is slightly corrosive. Steaming or a hot-water rinse is proper after washing for disinfection and as an aid to rapid drying. A mixture made by the addition of a tablespoonful of one of the wetting agents previously named, per pail of water, is good when used alone for farm-utensil washing. A home-prepared paste for removal of milk stone deposits can be made by adding to Italian 4F pumice enough of a 5 per cent solution of a weak acid such as citric, tartaric, or acetic to make a paste. The deposits may be removed by scouring with this abrasive paste, followed by thorough washing and rinsing. P.S.L.

## PHYSIOLOGY

384. **Some Factors Affecting the Resistance of Ejaculated and Epididymal Spermatozoa of the Boar to Different Environmental Conditions.** JOHN F. LASLEY AND RALPH BOGART, Dept. of Anim. Husb., Univ. of Missouri, Columbia, Mo. *Amer. Jour. Physiol.*, 141, No. 5: 619. July, 1944.

Secretions from the accessory glands (prostate, seminal vesicles, Cowper's and urethral glands) did not greatly influence the resistance to a cold shock or storage potentialities of boar epididymal spermatozoa. The degree of dilution, using several different diluters, had no influence upon the resistance of epididymal spermatozoa to a cold shock. Diluting suspensions of epididymal and ejaculated boar spermatozoa with egg yolk-phosphate buffer increased their resistance to a cold shock and their survival under storage conditions.

The resistance of spermatozoa to a cold shock and their ability to survive during storage varies with the place in the reproductive tract from which they are obtained. Spermatozoa from the head of the epididymis are very resistant and survive during storage for long periods, but their resistance and survival capacity decrease as the distance of their location from the testis increases until spermatozoa in the ejaculate have practically no resistance or storage potentialities. It is suggested, on the basis of the foregoing observations, that the reduction in resistance and storage potentialities of boar spermatozoa is associated with changes within the spermatozoa rather than with changes in their environment. D.E.

## MISCELLANEOUS

385. **Tin in the Dairy Industry.** JULIA BAXTER, Battelle Memorial Institute, Columbus, Ohio. *Natl. Butter and Cheese Jour.*, 35, No. 7: 68. July, 1944.

Although tin is a scarce, strategic metal now, new developments in



using it will favor its use after the war in the dairy industry. For example, electrolytic plating can apply economically, accurately and rapidly, definite and uniform coatings of any desired thickness to vats, cans, pipe-lines and tin plate. White bronze, a tin base material containing 35 to 50 per cent tin, and tin in combination with nickel offer new possibilities in coating copper base materials and steel. Tin with 8 per cent zinc gives a stiff foil which may be used for capping milk bottles. The life of tinned equipment can be extended by avoiding corrosive acid and alkali cleaners. W.V.P.

386. **A Family and an Industry.** PAUL H. MANDT, Olsen Publishing Co. Milwaukee, Wis. Natl. Butter and Cheese Jour., 35, No. 7: 16. July, 1944.

This is an interview with Loomis Burrell, whose grandfather, Jonathan Burrell, was a pioneer in the development of dairying in New York State nearly 150 years ago. The records of Loomis Burrell and his family show the importance of cheesemaking in those days, the factory problem, the adoption of English methods, and the gradual development of better mechanical devices and marketing methods. Dates of events and inventions, names of inventors, and cuts of early machinery are interesting features of this historical sketch. W.V.P.



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New York Association of Dairy and Milk Inspectors	United States Department of Agriculture

# ABSTRACTS OF LITERATURE

## BOOK REVIEWS

387. **Annual Review of Biochemistry. Volume 13.** Published by Annual Reviews, Inc., Stanford University P. O., Calif. 27 sections including indexes. 795 pages. \$5.00.

As in previous volumes, *Annual Review of Biochemistry* is the handbook whereby competent authors have the opportunity to review the recent developments in selected fields of biochemistry. The subjects of discussion and the authors are selected by an editorial board. In each of the reviews, the authors appraise, as well as review, the developments. Milk, being biologically complex, is related directly or indirectly to many of the subjects of discussion in Annual Reviews. Of the discussions of direct interest to those engaged in dairy research may be cited the following: Biological Oxidations and Reductions; Non-Oxidative Enzymes; Chemistry of: The Carbohydrates; the Lipids, the Proteins and Amino Acids. Metabolism of: Carbohydrate; Fat; Proteins and Amino Acids. Mineral Metabolism; Water and Fat Soluble Vitamins; Nutrition; Nutritional Deficiencies of Farm Animals (by Huffman and Duncan of Michigan State College). Other sections are reviews on Steroids, Biochemistry of Nucleic Acids, Purines, and Pyrimidines, Chemistry of Hormones, Biochemistry of Malignant Tissue, Alkaloids, Synthetic Drugs (Antispasmodics), Photo-Periodism in Plants, Chloroplast Pigments, Mineral Nutrition of Plants, Growth Regulating Substances in Plants, Biochemistry of Fungi, and Histochemistry. Annual Reviews is definitely the tool of the trained research worker, written in condensed, technical style.

K.G.W.

## BACTERIOLOGY

388. **The Rapid Identification of Mucoid Hemolytic Streptococci of Possible Epidemic Origin.** MAJOR ISADORE PILOT, M.C., A.U.S. Amer. Med. Assoc. Jour., 125, No. 15: 1037. Aug. 12, 1944.

A medium is described for the rapid isolation of mucoid strains of beta hemolytic streptococci (*Streptococcus epidemicus*) associated with milk-borne epidemics of sore throat and scarlet fever. Ascites fluid infusion blood agar has been used, but ascites fluid is not always available. Difco dehydrated brain heart infusion prepared with 1.75 per cent agar furnished a satisfactory base for moist plates with 5 per cent human blood added. In 12 to 24 hours, colonies appear large, mucoid, ameboid and confluent. Infusion agar prepared with various peptones failed to bring out the large mucoid growth. Isolation is followed by grouping and typing of the cultures.

Of recently reported milk-borne outbreaks of sore throat and scarlet fever, now traced to serologically specific types of hemolytic streptococci, type 3 accounted for three outbreaks, types 2 and 17 for others. These types appear to be sources of epidemic strains, but no mention is made of any mucoid property.

"The mucoid strains are particularly responsible for the bovine mastitis which is the source of epidemic milk-borne septic sore throat and scarlet fever. Experimentally, the mucoid strains ascend into the udder by simply smearing the teat and cause a similar mastitis. This aggressive property of the mucoid strains is further demonstrated by the frequency of their occurrence in otitis media, mastoiditis and meningitis complicating sore throat."

D.P.G.

## BUTTER

389. Importance of Copper in Certain Color Changes in Butter. R. V. HUSSONG AND B. W. HAMMER, Res. Lab., Sugar Creek Creamery Co., Danville, Ill. Food Res., 9, No. 4: 289. July-Aug., 1944.

Additional evidence is presented of the ability of copper contamination of cream to cause bleaching of butter accompanied by tallowy flavor; also, evidence that copper contamination of wrappers, even in isolated spots, may initiate bleaching and off-flavor, which will start adjacent to the spot and eventually spread to areas of the butter not contaminated with copper. The fact that, even when the butter is uniformly contaminated with copper, the bleaching begins at the surface is evidence of the importance of air as an accelerating agent in the reaction.

F.J.D.

## CHEESE

390. Some Factors Affecting the Behavior of Cheddar Cheese in Cooking. CATHERINE PERSONIUS, EDITH BOARDMAN, AND ANDRIANNA R. AUSHERMAN, Dept. of Home Econ., Univ. of Wis., Madison, Wis. Food Res., 9, No. 4: 304. July-Aug., 1944.

The authors present evidence indicating that Cheddar cheese improves in cooking quality with curing, the improvement being more rapid in cheese of high moisture content and in the case of curd cured in hermetically sealed cans as compared with daisies. The soluble protein of the cheese also increases with age, but there is no definite relationship between this factor and cooking quality. Low fat cheeses have poor cooking quality.

The blending of cheese with liquids is affected by the pH and ion content of the liquid. At pH 5.8 and over, good dispersion is obtained. At pH 4.0 to 5.0, the cheese tends to separate from the liquid in hard curd-like particles. High temperatures employed in preparation of cheese mixtures intensify the tendency of the cheese to mat, string, become tough and separate fat.

F.J.D.

391. **Salt Migration in Cheddar Cheese Curd and Its Effect on Moisture Content, pH and Bacterial Content.** W. H. HOECKER AND B. W. HAMMER, Iowa Agr. Expt. Sta., Ames, Iowa. Food Res., 9, No. 4: 278. July-Aug., 1944.

This study shows that cheese loses moisture rapidly at the surface of individual curd particles and in the interior of the particles during the first 15 hours of pressing. The greatest loss is at the surface. Losses after 15 hours are relatively small. The salt content of cheese is relatively high at the particle surface, at hooping time, and relatively low in the centers. During pressing, the salt decreases at the surface and increases at the center; and 24 hours after pressing, it is usually quite uniform throughout the particle. At milling, the pH values are essentially the same at the surface and the center of the unsalted curd particles. When hooped, the surface values are slightly higher and the values at the center slightly lower. During pressing, values at both places fall rapidly and gradually approach each other, until at 24 hours after pressing they are about equal. Changes in bacterial counts were not sufficiently definite in the study to be conclusive.

In Cheddar cheese ripened four months, the distribution of moisture and salt was found to be fairly uniform. F.J.D.

## CHEMISTRY

392. **Human-Milk Fat. 1. Component Fatty Acids.** T. P. HILDITCH AND M. L. MEARA, Dept. of Indus. Chem., Univ. of Liverpool, England. Biochem. Jour., 38, No. 1: 29-34. 1944.

A limited amount of early and late lactation human-milk fat and a larger amount of saponified human-milk fat were analyzed by ester-fractionation to determine the various fatty acids contained therein.

Their results show there is about an equal amount of saturated and unsaturated fatty acids in human-milk fat. Compared with cow-milk fat, it contains a relatively smaller percentage of the lower molecular weight fatty acids and a much larger percentage of the 18-carbon unsaturated fatty acid, this being unusually high for an animal fat. "Human-milk fat, in regard to its component acids, has more resemblance to a typical margarine fat-blend than to butterfat." A.O.C.

393. **Adsorption of Riboflavin by Lactose. Influence of Temperature.** ABRAHAM LEVITON, Bur. of Dairy Indus., U. S. Dept. Agr., Washington, D. C. Indus. and Engin. Chem., Indus. Ed., 36, No. 8: 744. Aug., 1944.

This work follows an earlier paper on the influence of concentration. A knowledge of the influence of temperature furnishes a basis for the production of adsorbates (milk sugar containing adsorbed riboflavin) as by-products in the manufacture of lactose. To utilize the productive capacity

of milk sugar plants for the manufacture of adsorbates, it is necessary to establish conditions for the controlled preparation of these adsorbates. The range of concentrations and temperatures studied include those which would be encountered in the manufacture of adsorbates from grain curd casein whey and are applicable to a wide range of operating conditions. In the commercial manufacture of lactose, even if all the riboflavin present in whey were adsorbed, a weak adsorbate would be obtained because the ratio between the quantity of lactose crystallizing from whey and the quantity of riboflavin in the whey is large. There is a critical riboflavin concentration beyond which the degree of adsorption rises sharply and increases linearly at 5° and 28° C. with increasing concentration. The data are considered primarily from the standpoint of their practical application, but a number of problems of both theoretical and practical interest are discussed.

B.H.W.

394. **Composition of Casein in Milk.** G. A. RAMSDELL AND E. O. WHITTIER, Div. of Dairy Res. Labs., Bur. of Dairy Indus., Agr. Res. Admin., U.S.D.A., Washington, D. C. *Jour. Biol. Chem.*, 154, No. 2: 413-419. 1944.

Heretofore the analyses reported for casein have been on the basis of the acid-precipitated product. The authors have employed a new method to separate the calcium caseinate-calcium phosphate complex from milk—by means of a supercentrifuge.

“In the literature there is no analysis of the elementary composition of casein obtained by our procedure, and that the results check, as a whole, surprisingly well with analyses of casein obtained by acid precipitation is interesting evidence that the changes in physical character through the action of acid are not accompanied by a pronounced alteration in its percentage elemental composition. However; the fact that the percentages we found for sulfur and phosphorous are somewhat higher than those obtained for casein repeatedly dissolved by alkali and precipitated by acid indicates that such treatment removes a portion of these elements from casein.”

The authors report the complex containing 4.80% tricalcium phosphate and 95.20% calcium caseinate; and this calcium caseinate having the following percentage composition: Calcium 1.18, Phosphorus 0.78, Nitrogen 15.34, Carbon 52.29, Hydrogen 6.919, Sulfur 0.762, and Oxygen (by difference) 22.73.

A.O.C.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

395. **Evaporated Milk. Current Comment.** ANONYMOUS. *Amer. Med. Assoc. Jour.*, 125, No. 12: 852. July 22, 1944.

Physicians are urged to avoid prescribing evaporated milk by named brands. Reference is made to a survey of the distribution of evaporated



milk in which it was shown that the shortage proved to be not a shortage of evaporated milk but of certain favorite brands. Less-well-known brands were available but not moving. Instead of advising against changing brands, physicians can do a service to their patients and their country by explaining to mothers that all brands of evaporated milk must meet federal standards. If the favorite or accustomed brand is not available, satisfactory results can be expected from the use of less familiar brands, provided consideration is given to whether the milk selected is irradiated or fortified with vitamin D. If the milk selected is not so fortified, supplementation of the diet or formula with this vitamin can be made separately. \* D.P.G.

396. **Retardation of Fat Autoxidation in Dried Milks.** M. B. WILLIAMSON, Res. Labs., S.M.A. Corp., Chagrin Falls, Ohio. Food Res., 9, No. 4: 298. July-Aug., 1944.

Several antioxidants, rice bran concentrate, Avenex, tocopherols, hydroxyquinone monobenzyl ether and 4,4-dehydroxydiphenyl ether, tested for their potency on mixed fats, were found to be effective when the fats were incorporated into skim milk powder. Thiourea, normally without effect in protecting fats, showed antioxygenic powers for fats combined with skim milk in the dry form. The authors show this to be due to the presence of water and discuss the theory of this action. F.J.D.

## DISEASE

397. **Lactic Acid: A Corrosive Poison.** E. GORDON YOUNG AND RALPH P. SMITH, Depts. of Biochem. and Path., Dalhousie Univ., Halifax, N.S. Amer. Med. Assoc. Jour., 125, No. 17: 1179-1181. Aug. 26, 1944. D.P.G.

398. **Mastitis.** E. C. McCULLOCH, A. A. SPIELMAN AND O. J. HILL. State Col. of Wash. Ext. Cir. 75. 4 pages. April, 1944.

A brief summary depicting, by means of cartoons and diagrams, the causes, spread, prevention, and control of mastitis. J.G.A.

## FEEDS AND FEEDING

399. **Vitamins D and A in Alfalfa Hay.** G. C. WALLIS. South Dakota Agr. Expt. Sta. Cir. 53. 12 pages. June, 1944.

Vitamin D develops as rapidly in small windrows as in the swath. Less vitamin D develops in large windrows than in the swath and small windrows, and still less in cocks. Most of the increase in cocks takes place in the outside layers.

Carotene losses are much less from the windrow than from the swath. Losses are still less from cocks.

Allowing the hay to wilt in the swath for a few hours and then raking it into small to medium sized windrows provides for rapid drying of the hay, encourages the development of as much vitamin D as the particular plants are capable of producing, and conserves a fair amount of carotene.

Finishing the curing in cocks after partial drying in the swath and windrow tends to conserve more of the carotene but takes more time and labor and provides less vitamin D.

Turning the windrows for the last half day or full day of curing increases the vitamin D content by about 100 International Units per pound as compared with unturned windrows.

The development of vitamin D in alfalfa hay by sunshine exposure is a comparatively slow process which continues gradually over a period of at least 6 or 8 days. The vitamin D content of hay could be increased by continuing the curing beyond the length of time necessary for proper drying, but the losses in carotene and other valuable properties would more than offset the gains.

Alfalfa hay probably varies between 300 and 1,000 International Units of vitamin D per pound, averaging about 500 to 600 units.

Alfalfa varies from time to time in the amount and rate of vitamin D development induced by sunshine exposure. The reasons for such variations are not known.

J.G.A.

#### 400. The Intensity of Feeding as Related to Milk Production. T. A.

BAKER AND A. E. TOMHAVE. Del. Agr. Expt. Sta. Bul. 248. 15 pages. June, 1944.

Five groups of Holstein cows were fed maintenance rations plus allowances of total digestible nutrients for milk production equal to 82.2, 105.3, 122.4, and 131 per cent of the Haecker standard, respectively. Substantial increases in production were obtained by feeding in excess of the Haecker standard. The amount of milk produced for each pound of total digestible nutrients fed was greatest at the lowest feeding level. The addition of a pound of nutrients produced the same increase in milk production at each higher level. For the most profitable milk production it can be conservatively recommended that the allowance of total digestible nutrients prescribed by the Haecker standard be increased by at least 20 per cent unless the cost of each added pound of total digestible nutrients is as great as the net cash return from 1.46 pounds of milk containing 4 per cent butterfat. Feeding digestible nutrients at different levels had no effect on the body weight or condition of the cows. There was no effect of feeding at different levels on butterfat percentage.

J.G.A.

401. **Feeding Peanut Meal and Hay.** W. J. SHEELY, R. B. BECKER, N. R. MEIRHOF, AND H. L. BROWN. Fla. Agr. Ext. Serv. Bul. 115. 15 pages, illus.

Detailed information on the composition of these Florida-grown products, together with specific directions for feeding them to beef cattle, dairy cattle, swine, and poultry.

J.G.A.

402. **Carotene Content of Alfalfa. Retention on Dehydration and Storage.** RALPH E. SILKER, W. G. SCHRENK, AND H. H. KING, Kans. Agr. Expt. Sta., Manhattan, Kans. Indus. and Engin. Chem., Indus. Ed., 36, No. 9: 831. Sept., 1944.

The effect of blanching, grinding, storage temperature and chemicals upon the stability of carotene in alfalfa was studied. Blanching the fresh alfalfa with steam prior to drying, thereby inactivating the enzymes, furnished complete protection for the carotene during the dehydration process. The grinding of fresh alfalfa caused a loss of carotene. The addition of a suitable antioxidant or addition of a chemical which was known to suppress enzyme activity furnished partial protection for the carotene during the dehydration. Diphenylamine and hydroquinone were the most effective antioxidants while thiourea and sodium cyanide helped to inactivate enzymes. The carotene content of alfalfa meal decreased as the temperature of storage increased. Storage at 3° C. of blanched and dehydrated alfalfa protected the carotene for long periods. There was a loss when the meal was removed from cold storage and allowed to stand at higher temperatures.

B.H.W.

## FOOD VALUE OF DAIRY PRODUCTS

403. **Riboflavin Content of Milk and Milk Products.** LOUISE DANIEL AND L. C. NORRIS, School of Nutr., Cornell Univ., Ithaca, N. Y. Food Res., 9, No. 4: 312. July-Aug., 1944.

Modified fluorometric and microbiological methods for determining the riboflavin content of 18 different dairy products were found to give results without significant differences, but on the whole somewhat lower than values reported in the literature where rat-assay methods were employed.

Average riboflavin values on the fresh basis for the products studied are presented in  $\mu\text{g./gm.}$  as follows: dried sweet cream buttermilk, 33.65; dried whey, 20.72; dried skim milk, spray process, 19.81, roller process, 18.81; dried whole milk, spray process, 15.44, roller process, 14.76; Cheddar cheese, 3.00; cream cheese, 1.87; liquid wholemilk, 1.77; liquid skim milk, 1.58; liquid buttermilk (cultured), 1.56; light cream, 1.47; liquid whey, 1.24; and butter, 0.367.

F.J.D.

## ICE CREAM

404. **General Points in the Handling of Sherbets.** B. I. MASUROVSKY, Res. Editor. *Ice Cream Trade Jour.*, 40, No. 8: 48. Aug., 1944.

Successful merchandising of bulk sherbets presents several problems. Consumers should not be forced to accept sherbets in place of, or in order to get, ice cream. Sherbets have been most popular when sold in factory-filled packages in combination with ice cream and in the form of novelties such as chocolate flavored "pop" containing milk sherbet. Strawberry, red raspberry, black raspberry, orange or pineapple "pops" have been popular. Vanilla, chocolate and other important ice cream flavors should not be used as sherbets in order to avoid confusion, or the possible substitution by ice cream dealers, or at the soda fountain. At least 20% of pure fruit material should be added to fruit ices and sherbets which are frozen with agitation. "Pops" and frozen confections are frozen without agitation. Overrun should be limited to not more than 40% for "pops" and not more than 50% for sherbets. Attention should also be given to sanitation. Care and attention in the production of high-quality sherbets now should aid in planning for postwar sales.

W.H.M.

405. **Mocha Flavor and Its Place in Ice Cream.** B. I. MASUROVSKY, Res. Editor. *Ice Cream Trade Jour.*, 40, No. 9: 52. Sept., 1944.

Mocha is a flavor created by a combination of chocolate and coffee. These two flavors may be added to the ice cream mix in extract form, or coffee extract may be added to a mild chocolate ice cream mix. If coffee is added to a chocolate mix, only about half the usual amount of chocolate ingredients should be present. The amount of coffee extract to use will vary from  $\frac{1}{2}$  to 1 ounce per gallon of ice cream mix. It should be free from chicory or other foreign flavoring material. Other points which should be observed in making Mocha-flavored ice cream are: (1) the sugar content should be less than that of chocolate ice cream or about the same as for vanilla ice cream. (2) The mix should contain no cereals or substitutes for milk solids. (3) It is desirable to increase the fat content 1 or 2 per cent above that of the standard vanilla mix.

Mocha and vanilla ice cream make a nice two-layer brick. Other recommended combinations are orange or strawberry sherbet, red raspberry sherbet and peach ice cream. When served at the fountain, Mocha ice cream should be topped with nuts and marshmallow rather than with chocolate sauce. In advertising the ice cream, the emphasis should be put on chocolate Mocha rather than coffee Mocha.

W.H.M.

## MILK

406. **Statistical Aspects of the Acidity Test.** H. BARKWORTH. Dairy Indus., 9, No. 1: 20. Jan., 1944.

The author points out the importance of controlling the amount of phenolphthalein used in the acidity test as this factor has been shown to have a greater effect on the result than any other single factor. The fact that the relationship is linear means that a given change in the amount of indicator will always cause the same amount of change of pH of the endpoint. By increasing the concentration of indicator from  $\log_{10}$  1.6990 (50 mmgms. per 100 ml. milk) to  $\log_{10}$  1.9031 (80 mmgms.) lowers the pH of the endpoint from 8.39 to 8.34. Similar changes in concentration will give corresponding changes in the pH of the endpoint. The correlation coefficient is large and therefore indicates a very close relationship between these two factors.

D.V.J.

407. **Effect of High Cell Content on Resazurin and Methylene Blue Tests.** C. S. MORRIS AND M. EDWARDS. Dairy Indus., 9, No. 2: 92. Feb., 1944.

It was found that the inclusion of high-cell-content milk into good quality bulk milk did not materially affect the results of the "Ten-Minute" Resazurin Test. However, in some cases when such milk was subjected to the Standard Routine Resazurin Test, a definite effect was noted so that the milk might be placed in the B or C categories. In some cases the addition of 5 per cent of high-cell-count milk to good quality, low-cell-count milk was sufficient to cause a noticeable change in the test.

The Methylene Blue Test was also found to be affected by the presence of high cell content milk in otherwise good quality milk. As little as 15 per cent of high-cell-count milk (from cows with no clinical symptoms of mastitis) can be sufficient for a failure on the Methylene Blue Test.

In some cases high-cell-count milk has the same resazurin reduction time as milk of normal cell content. In the majority of these cases, the cells present were mainly tissue cells.

The author points out that this work is only preliminary and in many ways incomplete.

D.V.J.

408. **Influence of Udder Cells on the Routine Resazurin Test, the Ten-Minute Resazurin Test and the Methylene Blue Test.** S. B. THOMAS AND D. A. BOWIE. Dairy Indus., 9, No. 5: 335. May, 1944.

Approximately 5,000 bulk herd samples were examined over a period of three years. Seventy per cent of the samples had cellular counts under

750,000 per ml., while 20 per cent had counts between 750,000 and 1,500,000. Eight per cent of the samples contained over 1,500,000 cells per ml.

Normal milk from herds with a low incidence of mastitis and counts under 750,000 cells per ml. were not degraded to categories B or C in the routine resazurin classification. Leucocyte activity alone was found responsible for the degradation to category B of 25 per cent of the samples with cell counts between 750,000 and 1,500,000. Milk infected with mastitis or containing a high proportion of late lactation milk was usually detected by the routine resazurin test. Sixty-five per cent of the samples containing over 1,500,000 cells per ml. were degraded to category B and 10 per cent to category C. Twenty-five per cent of these samples reduced methylene blue within  $5\frac{1}{2}$  hours.

D.V.J.

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